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Abstract

Objectives: Circulating 25-hydroxyvitamin D (25-OHD) is associated with improved cancer prognosis, yet it may be a surrogate marker for physical activity. Using data from the National Health and Nutrition Examination Survey (NHANES), we investigated the associations of leisure-time physical activity (LTPA) with circulating 25-OHD levels in cancer survivors, and determined whether associations differ by indoor and outdoor activity.

Design: Cross-sectional study.

Setting: The US National Health and Nutrition Examination Survey (NHANES).

Participants: Cancer survivors with available data on demographic information, measures of adiposity, smoking history, self-reported LTPA, circulating 25-OHD levels in five waves of NHANES (2001-2010).

Main outcomes measures: Circulating 25-OHD levels.

Results: Multiple linear regression and logistic regression models were used to evaluate the associations of self-reported LTPA with 25-OHD, adjusting for potential confounders. Due to the differences in LTPA measure, the analyses were conducted separately for 2001-2006, and 2007-2010 data. We further estimated associations by indoor and outdoor activity in the 2001-2006 data. There were 1,530 cancer survivors (mean age=60.5 years, mean BMI=28.6 kg/m^2). The prevalent cancer sites were breast (19.3%), prostate (18.8%), cervix (10.4%), and colon (8.6%). Compared to inactive cancer survivors, being physically active was associated with higher circulating 25-OHD levels; 9.19 nmol/L (95%CI: 5.24 to 13.14), and 9.12nmol/L (95%CI: 1.17 to 17.07) for 2001-2006 and 2007-2010 data, respectively. In the mutually adjusted model, outdoor

activity (5.72 nmol/L, 95%CI: 1.34 to 10.09), but not indoor activity (4.11 nmol/L, 95%CI: -0.87 to 9.08), was associated with statistically significant higher 25-OHD levels. The interaction between indoor and outdoor activities was not significant (P-value=0.12).

Conclusion: Physical activity, particularly outdoor activity is associated with higher 25-OHD levels in cancer survivors. Intervention in cancer survivors may consider including, and prioritizing outdoor activities.

Strengths and limitations of this study

- To the best of our knowledge, this is the first study to investigate the association of leisure-time physical activity (LTPA) with circulating 25-hydroxyvitamin D (25-OHD) levels in cancer survivors. We further compared associations by outdoor and indoor LTPA.
- The current study pooled data from cancer survivors in a nationally representative adult sample in the US.
- This study controlled for a range of factors that are known to affect circulating 25-OHD levels.
- Study limitations includes (1), the cross-sectional nature makes it impossible to determine a causal effect; (2) season, an important determinant of 25-OHD levels, was categorized into 2 (winter and summer, rather than winter, summer, fall and spring); (3) physical activity was self-reported.

Background

There are >15.5 million cancer survivors in the US and the number is expected to rise to 20 million by 2026.[1] Identifying factors, particularly modifiable factors, that improve prognosis and survival in this rapidly expanding demographic group is, therefore, a high priority.

There is emerging evidence that vitamin D status is associated with improved cancer prognosis and survival, particularly colorectal and breast cancers.[2] Circulating 25-hydroxyvitamin D (25-OHD) is the best indicator of overall vitamin D status because it has a long half-life, is unregulated by homeostatic systems in the body, and reflects total vitamin D from multiple determinants.[2] However, it has been suggested that circulating 25-OHD level may be a surrogate or biological marker for lifestyle factors that impact cancer prognosis, notably physical activity.[2-4] Physical activity, before and after cancer diagnosis, is associated with reduced mortality in cancer survivors,[5-7] although the underlying mechanisms are still being elucidated. In cancer-free population, leisure-time physical activity is associated with an increase in circulating 25-OHD levels; which is thought to reflect exposure to sunlight, a major determinant of circulating 25-OHD levels.[8] In support, studies have reported higher 25-OHD levels for the same amount of outdoor, compared to indoor physical activity,[9] although others have not.[10] Nevertheless, it has also been shown that physical activity and sun exposure may have independent effects on circulating 25-OHD levels, suggesting that indoor physical activity might be sufficient to increase circulating 25-OHD levels through its effect on 25-OHD metabolism, such as1,25-dihydroxyvitamin.[11-14]

To the best of our knowledge, no study has investigated the associations of physical activity with circulating 25-OHD levels in cancer survivors. Because physical activity declines after cancer diagnosis, findings in cancer-free population may not apply to cancer survivors. Using data from the National Health and Nutrition Examination Survey (NHANES), our objectives are to (i) investigate for the first time the associations of leisure-time physical activity with circulating 25-OHD levels in cancer survivors, (ii) determine whether associations differ by indoor and outdoor physical activity. Study findings could have implications for public health recommendations in cancer survivors because physical inactivity and vitamin D insufficiency are prevalent among cancer survivors,[15 16] and understanding the associations between physical activity and vitamin D could inform cancer survivorship care strategies.

Methods

Study Population

The National Health and Nutrition Examination Survey (NHANES) was designed to provide cross-sectional estimates on the prevalence of health, nutrition, and potential risk factors among the civilian non-institutionalized U.S. population up to 85 years of age.[17] In brief, NHANES surveys a nationally representative complex, stratified, multistage, probability clustered sample of about 5,000 participants each year in 15 counties across the country. The NHANES obtained approval from the National Center for Health Statistics Research Ethics Review Board and participants provided written consent.

We extracted demographic information, measures of adiposity, smoking history, self-reported leisure time physical activity, circulating 25-OHD levels, cancer diagnosis, and combined them into a single dataset for each data collection wave. Participants were considered as cancer survivors if they answered “yes” to the question “Have you ever been told by a doctor or other health professional that you had cancer or a malignancy of any kind?” We excluded participants who had non-melanoma skin cancer. This interview question was only given to males and females 20 years or older, subsequently restricted the analysed sample to adult cancer survivors. We created a single dataset for each wave of data from NHANES in 2001 to 2002, 2003 to 2004, 2005 to 2006, 2007 to 2008, and 2009 to 2010, and excluded those who were never diagnosed with cancer, and were pregnant.

Circulating 25-OHD levels

The process of blood collection is detailed in the NHANES Laboratory/Medical Technologist Procedures Manual.[18] Participants who received chemotherapy within last 4 weeks were excluded from blood collection. Blood samples were collected, processed, stored and shipped to University of Washington, Seattle for testing. The lab method measuring 25-OHD for 2007-2010 changed from 2005-2006 and earlier in NHANES, and has been described previously.[19] Briefly, circulating 25-OHD concentrations were measured at the National Center for Environmental health, CDC, Atlanta, GA using the DiaSorin RIA kit (Stillwater, MN) between 2001 and 2006. We converted the 25-OHD data in 2001-2006 using provided regression to equivalent 25-OHD measurement from a standardized liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, which was used in the analysis of 25-OHD in NHANES 2007-2010 data. This standardization procedure therefore ensures that 25-OHD data is comparable between 2001-2006 and 2007-2010.

Continuous 25-OHD data was used in linear regression models and categorized as low (<50 nmol/L) and high (≥50 nmol/L) 25-OHD in logistic regression models, based on definitions of vitamin D insufficiency.[20]

Socio-demographic characteristics

Socio-demographic characteristics including age, sex, race and ethnicity, and smoking status were extracted. Based on self-reported race and ethnicity, participants were classified into one of the three racial groups: Non-Hispanic White, Non-Hispanic Black,

and Hispanic and others. We classified participants into three groups: never smokers (did not smoke 100 cigarettes and do not smoke now), former smokers (smoked 100 cigarettes in life and do not smoke now), and current smokers (smoked 100 cigarettes in life and smoke now).

Body mass index (BMI)

Weight and height were measured at the time of physical examination in a mobile examination centre or in the participant's home. The measurements followed standard procedures and were carried out by trained technicians using standardized equipment. BMI was calculated as weight in kg/(height in meters)^2. We categorized study participants into standard BMI categories: underweight (<18.5kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0 – 29.9 kg/m^2), and obese (≥30.0 kg/m^2). For analytic purposes, we combined those who were underweight and those who had normal weight into 1 category (≤25 kg/m^2).

Season of blood draw

Blood samples were collected at the time of physical examination in a mobile examination or in the participants' home. Season of blood draw was determined from the documented month of physical examination. Months were reported in two groups: November 1st through April 30th, or May 1st through October 31st, and classified into winter or summer, respectively.[9]

Self-reported leisure-time physical activity (LTPA)

The assessment on self-reported physical activity for 2007-2010 changed from 2005-2006 and earlier. There is no conversion provided between two assessments, therefore analyses for LTPA were conducted separately in 2001 – 2006 data, and 2007 – 2010 data.

In 2001-2006 data, participants self-reported specific LTPA in the past 30 days from a list of 48 activities, that if they engaged in certain activities, and the frequencies and durations of these activities. Each activity was coded into a metabolic equivalent task (MET) score based on the 2011 Compendium of Physical Activities, a valid and globally used instrument to quantify the energy expenditure of physical activity in adults.[21] For each reported activity, MET-minutes per week (MET-min/week) were calculated by multiplying the MET value of each reported activity by the minutes spent in the activity per seven days. Overall LTPA was summarized as the total MET-minutes per week of all reported activities.[22] Cancer survivors were classified as inactive (zero MET-min/week), insufficiently active (<750 MET-min/week), and sufficiently active (≥750 MET-min/week) based on the standard definition.[22] In addition, we categorized each of the 48 listed activities into outdoor (e.g., walking, jogging, fishing) or indoor (e.g., aerobics, bowling, weights) activity. Activities that could be either indoor or outdoor (e.g., bicycling, swimming) were classified as indoor to ensure a conservative estimation of the associations between outdoor LTPA and 25-OHD. Both indoor and outdoor LTPA were summarized in MET-min/week, then classified as inactive (zero MET-min/week), insufficiently active (<450 MET-min/week), and sufficiently active (≥MET-min/week). We used 450 MET-min/week as the cut-off given is the minimal goal of weekly LTPA.[22]

In the 2007-2010 data, participants self-reported their daily activities, leisure time activities, and sedentary activities, using questions based on the Global Physical Activity Questionnaire (GPAQ).[23] Levels of LTPA were calculated as the minutes per week that participants reported participating in moderate-to-vigorous-intensity physical activity (MVPA). Participants reported the number of days and minutes spent in moderate recreational and vigorous recreational activities in a typical week, by answering questions “In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational activities?”, “Minutes vigorous recreational activities”, “In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational activities?”, and “Minutes moderate recreational activities”. We summarized the total number of minutes for both activities, where the number of minutes spent in vigorous-intensity physical activity were doubled and added to the number of minutes of moderate-intensity physical activity to approximately equivalent the MET value.[24] Cancer survivors were classified as inactive (zero min/week MVPA), insufficiently active (<150 min/week MVPA), and sufficiently active (≥150 min/week MVPA) based on the physical activity guidelines for cancer survivors.[25]

Statistical Analysis

Survey analysis procedures were used to account for the sample weights, stratification, and clustering of the complex sampling design to ensure nationally representative estimates. Information on socio-demographic characteristics, weight, height, season of blood draw, and self-reported LTPA was complete among cancer survivors who had available data on circulating 25-OHD levels. We calculated the descriptive statistics for participants' characteristics and LTPA categories by 25-OHD levels separately in 2001-2006 data, and 2007-2010 data. We summarized weighted means and standard errors for continuous variables, and weighted proportions for categorical variables.

We estimated linear associations between LTPA and 25-OHD in 2001-2006 data, and 2007-2010 data, respectively. The multiple linear regression models for LTPA were adjusted for age, sex, race, BMI, smoking status, and season of blood draw. In 2001-2006 data, we further estimated the linear associations between LTPA and 25-OHD separately by indoor and outdoor activities. In the multiple linear regression models, we simultaneously adjusted for both indoor and outdoor activities, provided they were significantly different (P value<0.001). We tested for differences between the indoor and outdoor effects by including both in the regression model and testing for interaction. We examined the normality of residuals by kernel density estimate and standardized normal probability plots for all the linear regression models. Using logistic regression models, we conducted similar analyses treating 25-OHD level as a binary outcome (<50 nmol/L vs. ≥50 nmol/L) to estimate the odds ratios of the associations between LTPA and 25-OHD in the 2001-2006 and 2007-2010 data.

All statistical significance was set at $p<0.05$. All statistical analyses were performed using Stata version 14.0 (STATA Corp., College Station, Texas, USA).

Results

Our study population consisted of 1,530 cancer survivors who had data on circulating 25-OHD levels. The most prevalent cancer sites were breast cancer (19.3%), prostate (18.8%), cervix (10.4%), and colon (8.6%). Participants' mean age at the time of baseline examination was 60.5 years, and their mean BMI was 28.6 kg/m^2. We observed statistically significant differences in circulating 25-OHD levels (<50 nmol/L vs. ≥50 nmol/L) for most characteristics, except for age, and sex (Tables 1 (2001-2006) and 2 (2007-2010)). Cancer survivors who were obese, Non-Hispanic Black, or smokers had lower 25-OHD levels than those who had normal weight, Non-Hispanic White or Hispanic and who were non-smokers, respectively.

[Insert Table 1 and Table 2]

Associations between LTPA and Circulating 25-OHD levels

Tables 3 and 4 summarized both the non-adjusted and adjusted associations between LTPA and circulating 25-OHD in linear regression and logistic regression models, respectively. Because LTPA measure differed between 2001-2006 and 2007-2010 and there is no conversion between the two, it is not possible to compare the findings between two study phrases directly. Cancer survivors who were sufficiently active had higher circulating 25-OHD levels than those who were inactive. This translated to 9.19 nmol/L (95% CI: 5.24 to 13.14) higher 25-OHD levels in 2001-2006 phase, and 9.12 nmol/L (95% CI: 1.17 to 17.07) higher in 2007-2010 phase in the multivariable-adjusted models. Compared to inactive, being insufficiently active was associated with 4.83 nmol/L (95% CI: 0.41 to 9.25) higher level of 25-OHD in 2001-2006 data. Furthermore,

the comprehensive data on a list of 48 activities collected in 2001-2006 allowed us to extend the analyses to compare between indoor and outdoor LTPA in relation to 25-OHD levels. In the non-adjusted models (Table 3), higher levels of indoor and outdoor LTPA both were associated with higher 25-OHD levels. However, in multivariable-adjusted models (that also mutually adjusted for indoor and outdoor LTPA), the association was only statistically significant among cancer survivors who engaged in outdoor LTPA (5.72 nmol/L, 95% CI: 1.34 to 10.09). The interaction between indoor and outdoor activities was not significant (P-value=0.12). Analyses using logistic regression models were supportive. Outdoor LTPA was lower in Non-Hispanic Black (69.2% inactive vs. 51.5% inactive among Non-Hispanic Whites, and 43.2% inactive among Hispanics) (Data not shown).

[Insert Table 3 and Table 4]

Discussion

We observed that being physically active was associated with higher circulating 25-OHD levels in cancer survivors. However, further analyses showed that the elevated 25-OHD levels were only statistically significant among cancer survivors who engaged in outdoor physical activity.

To the best of our knowledge, this is the first study to evaluate the associations of physical activity with circulating 25-OHD levels in cancer survivors. Our findings are, however, similar to what has been reported among non-cancer participants enrolled in NHANES (1988-1994).[9] Scargg and Camargo reported a 9.6 nmol/L increase in 25-OHD levels among participants who engaged in outdoor LTPA compared to those who did not engage in outdoor LTPA. The increase in 25-OHD levels associated with outdoor LTPA is higher than what we observed in our study population (5.72 nmol/L higher 25-OHD). This could be due to the different ways LTPA was categorized. The most active group in their study translates to participating daily in outdoor activity, whilst only 5.6% (weighted proportion) of cancer survivors in our sample achieved this physical activity level. To compare at an equivalently active level, our findings of a 5.72 nmol/L increase in cancer survivors is similar to 6.1 nmol/L higher 25-OHD level in individuals who were at a similar activity level (engaged in 13-30 times outdoor LTPA per month) reported by Scargg and Camargo.[9] A more recent analysis using NHANES 2003-2006 data reported increasing level of 25-OHD associated with higher level of objectively measured moderate-to-vigorous physical activity, but the association was not stronger for outdoor LTPA compared to indoor when using self-reported data.[10]

It is unclear whether physical activity has direct or indirect effects on 25-OHD levels. Sun exposure is the major determinant of circulating 25-OHD levels, hence, it is possible that physical activity may indirectly impact 25-OHD levels through increased sun exposure associated with outdoor activity[26] among active individuals; yet few studies have measured activities specifically to outdoor, or able to adjusted for sun exposure.[9 10 27 28] On the other hand, physical activity may directly impact 25-OHD metabolism. Zittermann and colleagues[11] reported higher calcium absorption rates and plasma calcritrol levels in exercise-trained young men compared to age-matched sedentary controls. Similarly, in a small study, young males who underwent muscle-building exercise (indoor) for at least 1 year had higher circulating 25-OHD, Gla-protein, and 1,25-dihydroxyvitamin levels compared to age-matched controls who received constant daily diet same as the exercise group.[13] However, whether this mechanism operates in cancer survivors is unclear, because of the physiological, biological and behavioral alterations associated with cancer, and cancer treatment.[25]

We observed statistically significant higher circulating 25-OHD levels associated with outdoor, but not with indoor, LTPA in the mutually adjusted model. Nevertheless, no statistically significant interaction between indoor and outdoor LTPA was observed. It is likely that LTPA influence 25-OHD via multiple pathways, possibly both an indirect effect due to sun exposure and a direct impact on 25-OHD metabolism. However this warrants further investigation using precise measures of physical activity[29] and taking into consideration sun exposure, seasonality, and other vitamin D metabolites.

The main strength of this analysis is pooling cancer survivors from a nationally representative adult sample in the US. We aggregated five waves' data and achieved a fairly sizeable sample. In addition, we controlled for a range of factors that are known to affect the circulating 25-OHD levels. Further, we were able to compare associations of LTPA with 25-OHD by outdoor and indoor LTPA, thereby providing further insights on the associations of LTPA with 25-OHD levels.

There are a number of limitations to this study. First, the cross-sectional nature makes it impossible to determine a causal effect. Second, season, an important determinant of 25-OHD levels, was only available in two categories. Solar radiation, required for skin to synthesize vitamin D, is weaker in winter compared to summer. However, there were no statistically significantly differences between winter (Southern states) and summer (Northern states) 25-OHD levels in our study population, probably owing to the timing of blood collection in each region. The NHANES study collected blood samples in the Southern states during winter, and in the Northern states during summer. Third, we were not able to conduct analyses stratified by cancer type or time since diagnosis because of the limited number of individual cancers. Finally, physical activity was self-reported. However, any bias arising from this is likely to be non-differential.

Our findings of an association between LTPA and 25-OHD, that was stronger for outdoor LTPA compared to indoor LTPA has implications for public health recommendations in cancer survivors. Although the casual relationship of 25-OHD with cancer survival is yet unclear, strong evidence supports the benefits of physical activity

in improved cancer survival and the quality of life during survival.[29 30] Our findings suggest that 25-OHD might be a surrogate marker of physical activity that accounts for the direct and indirect effects of LTPA, particularly outdoor. The proportion of cancer survivors in NHANES who did not engage in any LTPA was high, especially in the 2007-2010 (53.3%) compared to the 2001-2006 wave (38.3%). This observed decline in LTPA might be attributed to the difference in measures and may not reflect an actual change in LTPA levels, i.e. the 2001-2006 measure is comprised of 48 activity items whilst the 2007-2010 measure queries general physical activity participation. In fact, an increase in the physical activity level in the US population from 2001 to 2011 has been reported from the BRFSS data,[31] though this trend may not hold true in cancer survivors. Guidelines from the American Cancer Society[25] and American College of Sports Medicine[32] suggest that cancer survivors should follow the physical activity guidelines for Americans with specific exercise programming adaptations based on disease- and treatment-related adverse effects. However, physical activity levels in these populations are critically low during and after treatment.[33] Effort in designing physical activity interventions specifically to cancer survivors may consider including and prioritizing outdoor activities with the potentially benefits of sun exposure. Notably, given the well-documented differences in cancer prognosis between non-Hispanic Blacks and other racial groups, and the emerging associations of vitamin D with cancer prognosis, physical activity interventions incorporating outdoor activities might be particularly important for cancer survival among non-Hispanic Blacks.

In conclusion, physical activity, particularly outdoor physical activity is associated with higher 25-OHD levels in cancer survivors. This adds to the potential health benefits of being physically active. Non-Hispanic Black cancer survivors, who are more likely to have vitamin D deficiency, were less likely to engage in outdoor LTPA. Because of the established survival advantage associated with physical activity, and the emerging role of vitamin D in cancer prognosis, physical activity interventions in cancer survivors may consider including, and prioritizing outdoor activities.

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Ethics approval: National Center for Health Statistics Research Ethics Review Board Approval.

Contributors: LY and ATT conceived and designed study, analysed and interpreted data, drafted and reviewed manuscript.

Data sharing statement: The NHNAES data are publically available at https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

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Table 1. Socio-demographic Characteristics and Leisure Time Physical Activity of Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2006), by Circulating 25-OHD levels (n=793)

		Overall	Circulating 25-OHD <50 nmol/L	>=50 nmol/L	P-value
2001-2006	N	791	258	533	
Age (year)	Mean (s.e.)	60.3 (0.6)	60.2 (1.0)	60.1 (0.8)	0.93
BMI					0.001
<18.5	%	1.9	22.2	77.8	
18.5 – 24.9	%	32.7	26.0	74.0	
25.0 – 29.9	%	32.1	18.2	81.8	
≥ 30	%	33.3	37.6	62.4	
Season					0.06
Winter (November to April)	%	34.3	33.4	66.6	
Summer (May to October)	%	65.7	24.1	75.9	
Sex					0.63
Male	%	32.7	26.0	74.0	
Female	%	67.3	28.0	72.0	
Race					<.001
Non-Hispanic white	%	86.1	22.8	77.2	
Non-Hispanic black	%	6.6	67.0	33.0	
Hispanic and other	%	7.3	44.8	55.2	
Smoking					0.02
Never smoked	%	39.1	23.8	76.2	
Former smoker	%	39.8	25.3	74.7	
Current smoker	%	21.1	37.6	62.4	
Leisure time physical activity (LTPA)					<.001
Inactive	%	38.2	37.7	62.3	
Insufficiently Active	%	33.0	25.6	74.4	
Sufficiently Active	%	28.8	15.5	84.5	
Indoor LTPA					0.02
Inactive	%	61.7	32.0	68.0	
Insufficiently Active	%	18.2	20.3	79.7	
Sufficiently Active	%	20.1	19.2	80.8	
Outdoor LTPA					<.001
Inactive	%	52.0	35.4	64.6	
Insufficiently Active	%	22.0	19.7	80.3	
Sufficiently Active	%	26.0	17.6	82.4	

Table 2. Socio-demographic Characteristics and Leisure Time Physical Activity of Cancer Survivors Aged 20 years or Older from the NHANES (2007- 2010), by Circulating 25-OHD level (n=737)

		Overall	Circulating 25-OHD <50 nmol/L	>=50 nmol/L	P-value
2007-2010*	N	737	206	531	
Age (year)	Mean (s.e.)	60.8 (0.7)	58.7 (1.3)	61.4 (0.8)	0.08
BMI					0.04
<18.5	%	2.0	22.3	77.7	
18.5 – 24.9	%	27.2	17.7	82.3	
25.0 – 29.9	%	34.0	17.7	82.3	
≥ 30	%	36.8	28.0	72.0	
Season					0.1
Winter (November to April)	%	32.6	25.4	74.6	
Summer (May to October)	%	67.4	19.8	80.2	
Sex					0.03
Male	%	37.8	17.1	82.9	
Female	%	62.2	24.3	75.7	
Race					<.001
Non-Hispanic white	%	82.6	15.2	84.8	
Non-Hispanic black	%	8.2	54.3	45.7	
Hispanic and other	%	9.2	49.9	50.1	
Smoking					0.01
Never smoked	%	47.5	22.1	77.9	
Former smoker	%	35.1	16.0	84.0	
Current smoker	%	17.4	31.5	68.5	
Leisure time physical activity (LTPA)					0.009
Inactive	%	53.3	28.1	71.9	
Insufficiently Active	%	16.6	17.5	82.5	
Sufficiently Active	%	30.1	12.4	87.6	

Table 3. Associations between Leisure-Time Physical Activity and Circulating 25-OHD level from Unadjusted and Adjusted Multiple Linear Regression models among Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2010).

2001-2006* (n=793)		Circulating 25-OHD (nmol/L)			
		Unadjusted linear regression		Adjusted multiple linear regression†	
		Beta-coefficient (95% CI)	P-value	Beta-coefficient (95% CI)	P-value
Model 1:	Leisure time physical activity (LTPA)				
	Inactive	reference		reference	
	Insufficiently Active	7.36 (2.65 to 12.07)	0.003	4.83 (0.41 to 9.25)	0.03
	Sufficiently Active	12.16 (7.29 to 17.04)	<.001	9.19 (5.24 to 13.14)	<.001
	P for trend		<.001		<.001
Model 2:	Outdoor physical activity				
	Inactive	reference		reference	
	Insufficiently Active	9.10 (5.15 to 13.04)	<.001	6.69 (2.52 to 10.87)	0.002
	Sufficiently Active	8.84 (4.16 to 13.52)	<.001	5.72 (1.34 to 10.09)	0.01
	P for trend		<.001		0.007
	Indoor physical activity				
	Inactive	reference		reference	
	Insufficiently Active	3.15 (-1.63 to 7.94)	0.2	-0.69 (-4.57 to 3.18)	0.72
	Sufficiently Active	8.22 (2.50 to 13.93)	0.006	4.11 (-0.87 to 9.08)	0.10
	P for trend		0.004		0.11
2007-2010* (n=737)		Circulating 25-OHD (nmol/L)			
		Unadjusted linear regression		Adjusted multiple linear regression†	
		Beta-coefficient (95% CI)	P-value	Beta-coefficient (95% CI)	P-value
Model 3:	Leisure time physical activity (LTPA)				
	Inactive	reference		reference	
	Insufficiently Active	8.80 (-2.67 to 20.26)	0.13	7.45 (-2.74 to 17.64)	0.15
	Sufficiently Active	12.04 (5.24 to 18.84)	0.001	9.12 (1.17 to 17.07)	0.03
	P for trend		0.001		0.02

*Leisure-time physial activity (LTPA) data analyzed separately due to the changes in self-reported LTPA measures from wave 2005 - 2006 to 2007-2008.

†Adjusted for age, sex, race, body mass index, and smoking status.

Table 4. Associations between Leisure-Time Physical Activity and Circulating 25-OHD level from Unadjusted and Adjusted Logistic Regression models among Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2010).

2001-2006*	Circulating 25-OHD >=50 nmol/L (n=534)			
Reference:	Unadjusted logistic regressions		Adjusted multiple logistic regressions†	
Circulating 25-OHD <50 nmol/L (n=259)	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Model 1: Leisure time physical activity (LTPA)				
Inactive	reference		reference	
Insufficiently Active	1.73 (1.05 to 2.90)	0.03	1.46 (0.86 to 2.49)	0.16
Sufficiently Active	3.30 (2.54 to 5.32)	<.001	2.90 (1.84 to 4.58)	<.001
P for trend		<.001		<.001
Model 2: Outdoor physical activity				
Inactive	reference		reference	
Insufficiently Active	2.23 (1.38 to 3.61)	0.002	1.81 (1.11 to 2.96)	0.02
Sufficiently Active	2.56 (1.45 to 4.52)	0.002	2.11 (1.16 to 3.80)	0.01
P for trend		0.001		0.009
Indoor physical activity				
Inactive	reference		reference	
Insufficiently Active	1.85 (0.98 to 3.46)	0.06	1.40 (0.71 to 2.77)	0.32
Sufficiently Active	1.98 (1.17 to 3.35)	0.01	1.47 (0.84 to 2.56)	0.17
P for trend		0.006		0.14
2007-2010*	Circulating 25-OHD >=50 nmol/L (n=531)			
Reference	Unadjusted logistic regressions		Adjusted multiple logistic regressions†	
Circulating 25-OHD <50 nmol/L (n=206)	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Model 3: Leisure time physical activity (LTPA)				
Inactive	reference		reference	
Insufficiently Active	1.84 (0.89 to 3.81)	0.1	1.92 (0.90 to 4.11)	0.09
Sufficiently Active	2.76 (1.30 to 5.87)	0.01	2.26 (1.07to 4.77)	0.03
		0.008		0.03

*Leisure-time physial activity (LTPA) data analyzed separately due to the changes in self-reported LTPA measures from wave 2005 - 2006 to 2007-2008.

†Adjusted for age, sex, race, body mass index, and smoking status.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (page 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 5)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 6)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 6)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (Page 6)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 7-10)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 7-10)
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at (Page 6)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page 7-10)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 11-12)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 11)
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (Page 11)
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Page 13)
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 13)
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures (Page 13)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Page 13-14)
		(*b*) Report category boundaries when continuous variables were categorized (Page 7-10)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Page 14)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 15)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 17)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 15-16)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 17)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 20)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Leisure-time physical activity and circulating 25-hydroxyvitamin D levels in cancer survivors, a cross-sectional analysis using data from the National Health and Nutrition Examination Survey

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Title: Leisure-time physical activity and circulating 25-hydroxyvitamin D levels in cancer survivors, a cross-sectional analysis using data from the National Health and Nutrition Examination Survey

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Text: 3,875 words

Abstract

Objectives: Circulating 25-hydroxyvitamin D (25-OHD) is associated with improved cancer prognosis in some studies, yet it may be a surrogate marker for physical activity. Using data from the National Health and Nutrition Examination Survey (NHANES), we investigated the associations of leisure-time physical activity (LTPA) with circulating 25-OHD levels in cancer survivors, and determined whether associations differ by indoor and outdoor activity.

Design: Cross-sectional study.

Setting: The US National Health and Nutrition Examination Survey (NHANES).

Participants: Cancer survivors with available data on demographic information, measures of adiposity, smoking history, self-reported LTPA, circulating 25-OHD levels in five waves of NHANES (2001-2010).

Main outcomes measures: Circulating 25-OHD levels.

Results: Multivariable linear regression and logistic regression models were used to evaluate the associations of self-reported LTPA with 25-OHD, adjusting for potential confounders. Due to the differences in LTPA measure, the analyses were conducted separately for 2001-2006, and 2007-2010 data. We further estimated associations by indoor and outdoor activity in the 2001-2006 data. There were 1,530 cancer survivors (mean age=60.5 years, mean BMI=28.6 kg/m^2). The prevalent cancer sites were breast (19.3%), prostate (18.8%), cervix (10.4%), and colon (8.6%). Compared to inactive cancer survivors, being physically active was associated with higher circulating 25-OHD levels (8.07 nmol/L, 95%CI: 4.63 to 11.52) for 2001-2006 data. In the mutually adjusted model, higher outdoor activity (5.83 nmol/L, 95%CI: 1.64 to 10.01), but not indoor

activity (2.93 nmol/L, 95%CI: -1.80 to 7.66), was associated with statistically significant higher 25-OHD levels. The interaction between indoor and outdoor activities was, however, not significant (P-value=0.29).

Conclusion: Physical activity, particularly outdoor activity is associated with higher 25-OHD levels in cancer survivors. In view of the possible beneficial effects of vitamin D on cancer prognosis, engaging in outdoor physical activity could provide clinically meaningful increases in 25-OHD levels among cancer survivors.

Strengths and limitations of this study

- To the best of our knowledge, this is the first study to investigate the association of leisure-time physical activity (LTPA) with circulating 25-hydroxyvitamin D (25-OHD) levels in cancer survivors. We further compared associations by outdoor and indoor LTPA.
- The current study pooled data from cancer survivors in a nationally representative adult sample in the US.
- This study controlled for a range of factors that are known to affect circulating 25-OHD levels.
- Study limitations includes (1), the cross-sectional nature makes it impossible to determine a causal effect; (2) season, an important determinant of 25-OHD levels, was categorized into 2 (winter and summer, rather than winter, summer, fall and spring); (3) physical activity was self-reported.

Background

There are >15.5 million cancer survivors in the US and the number is expected to rise to 20 million by 2026.[1] Identifying factors, particularly modifiable factors, that improve prognosis and survival in this rapidly expanding demographic group is, therefore, a high priority.

There is emerging evidence that vitamin D status is associated with improved cancer prognosis and survival, particularly colorectal and breast cancers.[2-5] Circulating 25-hydroxyvitamin D (25-OHD) is the best indicator of overall vitamin D status because it has a long half-life, is unregulated by homeostatic systems in the body, and reflects total vitamin D from multiple determinants.[6-9] However, it has been suggested that circulating 25-OHD level may be a surrogate or biological marker for lifestyle factors that impact cancer prognosis, notably physical activity.[2 10 11] Physical activity, before and after cancer diagnosis, is associated with reduced mortality in cancer survivors,[12-14] although the underlying mechanisms are still being elucidated. In cancer-free population, leisure-time physical activity is associated with an increase in circulating 25-OHD levels; which is thought to reflect exposure to sunlight, a major determinant of circulating 25-OHD levels.[15] In support, studies have reported higher 25-OHD levels for the same amount of outdoor, compared to indoor physical activity,[16] although others have not.[17] Nevertheless, it has also been shown that physical activity and sun exposure may have independent effects on circulating 25-OHD levels, suggesting that indoor physical activity might be sufficient to increase circulating 25-OHD levels through its effect on 25-OHD metabolism, such as1,25-dihydroxyvitamin.[18-21]

To the best of our knowledge, no study has investigated the associations of physical activity with circulating 25-OHD levels in cancer survivors. Because physical activity declines after cancer diagnosis, findings in cancer-free population may not apply to cancer survivors. Using data from the National Health and Nutrition Examination Survey (NHANES), our objectives are to (i) investigate the associations of leisure-time physical activity with circulating 25-OHD levels in cancer survivors, (ii) determine whether associations differ by indoor and outdoor physical activity. Study findings could have implications for public health recommendations in cancer survivors because physical inactivity and vitamin D insufficiency are prevalent among cancer survivors.[22 23]

Methods

Study Population

The National Health and Nutrition Examination Survey (NHANES) was designed to provide cross-sectional estimates on the prevalence of health, nutrition, and potential risk factors among the civilian non-institutionalized U.S. population up to 85 years of age.[24] In brief, NHANES surveys a nationally representative complex, stratified, multistage, probability clustered sample of about 5,000 participants each year in 15 counties across the country. The NHANES obtained approval from the National Center for Health Statistics Research Ethics Review Board and participants provided written consent.

We extracted demographic information, measures of adiposity, smoking history, self-reported leisure time physical activity, circulating 25-OHD levels, cancer diagnosis, and combined them into a single dataset for each data collection wave. Participants were considered as cancer survivors if they answered “yes” to the question “Have you ever been told by a doctor or other health professional that you had cancer or a malignancy of any kind?” We excluded participants who had non-melanoma skin cancer. This interview question was only given to males and females 20 years or older, subsequently restricted the analysed sample to adult cancer survivors. We created a single dataset for each wave of data from NHANES in 2001 to 2002, 2003 to 2004, 2005 to 2006, 2007 to 2008, and 2009 to 2010, and excluded those who were never diagnosed with cancer, or were pregnant. (Figures 1 and 2)

Circulating 25-OHD levels

The process of blood collection is detailed in the NHANES Laboratory/Medical Technologist Procedures Manual.[25] Participants who received chemotherapy within last 4 weeks were excluded from blood collection in the NHANES study. Blood samples were collected, processed, stored and shipped to University of Washington, Seattle for testing. The lab method measuring 25-OHD for 2007-2010 changed from 2005-2006 and earlier in NHANES, and has been described previously.[26] Briefly, circulating 25-OHD concentrations were measured at the National Center for Environmental health, CDC, Atlanta, GA using the DiaSorin RIA kit (Stillwater, MN) between 2001 and 2006. We converted the 25-OHD data in 2001-2006 using provided regression to equivalent 25-OHD measurement from a standardized liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, which was used in the analysis of 25-OHD in NHANES 2007-2010 data. This standardization procedure therefore ensures that 25-OHD data is comparable between 2001-2006 and 2007-2010.

Socio-demographic characteristics

Socio-demographic characteristics including age, sex, race and ethnicity, and smoking status were extracted. Based on self-reported race and ethnicity, participants were classified into one of the three racial groups: Non-Hispanic White, Non-Hispanic Black, and Hispanic and others. We classified participants into three groups: never smokers (did not smoke 100 cigarettes and do not smoke now), former smokers (smoked 100 cigarettes in life and do not smoke now), and current smokers (smoked 100 cigarettes in life and smoke now).

Body mass index (BMI)

Weight and height were measured at the time of physical examination in a mobile examination centre or in the participant's home. The measurements followed standard procedures and were carried out by trained technicians using standardized equipment. BMI was calculated as weight in kg/(height in meters)^2. We categorized study participants into standard BMI categories: underweight (<18.5kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0 – 29.9 kg/m^2), and obese (≥30.0 kg/m^2). For analytic purposes, we combined those who were underweight and those who had normal weight into 1 category (≤25 kg/m^2).

Season of blood draw

Blood samples were collected at the time of physical examination in a mobile examination center (MEC) or in the participants' home. Season of blood draw was determined from the documented month of physical examination. Months were reported in two groups: November 1st through April 30th, or May 1st through October 31st, and classified into winter or summer, respectively.[16]

Dietary Vitamin D supplement use

Information on dietary vitamin D supplement was retrieved from the 30-day Dietary Supplement dataset in the 2001-2006 and 2007-2010 data. In the 2001-2006 dataset, we obtained data on individual product for participants who reported taking vitamin supplement, and linked to the Dietary Supplements Ingredient Database.[27] Products'

ingredient that contained Vitamin D were aggregated for each participant, and then categorized into a binary variable (yes/no) for dietary vitamin D supplement use assessment. In 2007-2010 data, participants' total dietary supplement use data was available, thus, was used to determine their dietary vitamin D supplement use (yes/no).

Self-reported leisure-time physical activity (LTPA)

The assessment on self-reported physical activity for 2007-2010 changed from 2005-2006 and earlier. There is no conversion provided between two assessments, therefore analyses for LTPA were conducted separately for the 2001 – 2006, and 2007 – 2010 data.

In the 2001-2006 data, participants self-reported specific LTPA in the past 30 days from a list of 48 activities, that if they engaged in certain activities, and the frequencies and durations of these activities. Each activity was coded into a metabolic equivalent task (MET) score based on the 2011 Compendium of Physical Activities, a valid and globally used instrument to quantify the energy expenditure of physical activity in adults.[28] For each reported activity, MET-minutes per week (MET-min/week) were calculated by multiplying the MET value of each reported activity by the minutes spent in the activity per seven days. Overall LTPA was summarized as the total MET-minutes per week of all reported activities.[29] Cancer survivors were classified as inactive (zero MET-min/week), insufficiently active (<750 MET-min/week), and sufficiently active (≥750 MET-min/week) based on the standard definition.[29] In addition, we categorized each of the 48 listed activities into outdoor (e.g., walking, jogging, fishing) or indoor (e.g.,

aerobics, bowling, weights) activity. Activities that could be either indoor or outdoor (e.g., bicycling, swimming) were classified as indoor to ensure a conservative estimation of the associations between outdoor LTPA and 25-OHD. Both indoor and outdoor LTPA were summarized in MET-min/week, then classified as inactive (zero MET-min/week), insufficiently active (<450 MET-min/week), and sufficiently active (≥450 MET-min/week). We used 450 MET-min/week as the cut-off given is the minimal goal of weekly LTPA.[29]

In the 2007-2010 data, participants self-reported their daily activities, leisure time activities, and sedentary activities, using questions based on the Global Physical Activity Questionnaire (GPAQ).[30] Levels of LTPA were calculated as the minutes per week that participants reported participating in moderate-to-vigorous-intensity physical activity (MVPA). Participants reported the number of days and minutes spent in moderate recreational and vigorous recreational activities in a typical week, by answering questions “In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational activities?”, “Minutes vigorous recreational activities”, “In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational activities?”, and “Minutes moderate recreational activities”. We summarized the total number of minutes for both activities, where the number of minutes spent in vigorous-intensity physical activity were doubled and added to the number of minutes of moderate-intensity physical activity to approximately equivalent the MET value.[31] Cancer survivors were classified as inactive (zero min/week MVPA), insufficiently active (<150 min/week MVPA), and sufficiently active (≥150 min/week MVPA) based on the physical activity guidelines for cancer survivors.[32]

Statistical Analysis

Survey analysis procedures were used to account for the sample weights (MEC exam weight), stratification, and clustering of the complex sampling design to ensure nationally representative estimates. Information on socio-demographic characteristics, weight, height, season of blood draw, and self-reported LTPA was complete among cancer survivors who had available data on circulating 25-OHD levels. We calculated the descriptive statistics for participants' characteristics and LTPA categories by 25-OHD levels in quintiles separately in 2001-2006 data, and 2007-2010 data. We summarized weighted means and standard errors for continuous variables, and weighted proportions for categorical variables.

We estimated linear associations between LTPA and 25-OHD levels in both 2001-2006 and 2007-2010 data. The multivariable linear regression models for LTPA were adjusted for age, sex, race, BMI, smoking status, and season of blood draw. In the 2001-2006 data, we further estimated the linear associations between LTPA and 25-OHD separately by indoor and outdoor activities. Chi-square test indicated significant difference (P-value<0.001) between indoor and outdoor activities. In the multivariable linear regression models, we simultaneously adjusted for both activities. We tested for differences between the indoor and outdoor effects by including both in the regression model and testing for interaction. We examined the normality of residuals by kernel density estimate and standardized normal probability plots for all the linear regression models. Continuous 25-OHD data was categorized as low (<50 nmol/L) and high (≥50 nmol/L) 25-OHD based on definitions of vitamin D insufficiency.[30]

To calculate the prevalence ratios (PRs) of high 25-OHD level (≥50 nmol/L) across LTPA categories, we first calculated prevalence odds ratios (PORs) for each category in multivariable logistic regression models. Since the PORs do not approximate the PRs for common outcome (25-OHD ≥50 nmol/L), we used the baseline prevalence to correct the PORs and 95% confidence intervals based on existing method to obtain reliable PRs estimates.[33] We further conducted following sensitivity analyses: 1) using BMI as a continuous variable in the regression models; 2) stratification by BMI category; 3) classifying activities that could be either indoor or outdoor (e.g., bicycling, swimming) as outdoor activities; 4) classifying activities that could be either indoor or outdoor (e.g., bicycling, swimming) as half-half (MET-min/week) to indoor and outdoor activities.

All statistical significance was set at $p<0.05$. All statistical analyses were performed using Stata version 14.0 (STATA Corp., College Station, Texas, USA).

Results

Our study population consisted of 1,530 cancer survivors who had data on circulating 25-OHD levels. The most prevalent cancer sites were breast cancer (19.3%), prostate (18.8%), cervix (10.4%), and colon (8.6%). Participants' mean age at the time of baseline examination was 60.5 years, and their mean BMI was 28.6 kg/m^2. Circulating 25-OHD levels were signifincalty higher among those who reported dietary vitamin D supplement use than those who did not in both 2001-2006 (68.82 vs 56.74 nmol/L, p<.001) and 2007-2010 data (83.73 vs 60.88 nmol/L, p<.001). We observed statistically significant differences in circulating 25-OHD levels for most characteristics, except for age, and sex (Tables 1 (2001-2006) and 2 (2007-2010)). Cancer survivors who were obese, Non-Hispanic Black, or smokers had lower 25-OHD levels than those who had normal weight, Non-Hispanic White/Hispanic and were non-smokers, respectively.

[Insert Table 1 and Table 2]

Associations between LTPA and Circulating 25-OHD levels

Tables 3 and 4 summarize both the non-adjusted and adjusted associations between LTPA and circulating 25-OHD in linear regression and logistic regression models, respectively. Because LTPA measure differed between 2001-2006 and 2007-2010 and there is no conversion between the two, it is not possible to compare the findings between two study phrases directly. Cancer survivors who were sufficiently active had higher circulating 25-OHD levels than those who were inactive in univariate analyses, and these findings were maintained in multivariable analyses in the 2001-2006, but not the 2007-2010 data. This translated to 8.07 nmol/L (95% CI: 4.63 to 11.52) higher 25-

OHD levels in 2001-2006 phase in the multivariable-adjusted models. Furthermore, the comprehensive data on a list of 48 activities collected in 2001-2006 allowed us to extend the analyses to compare between indoor and outdoor LTPA in relation to 25-OHD levels. In the non-adjusted models (Table 3), higher levels of indoor and outdoor LTPA both were associated with higher 25-OHD levels. However, in multivariable-adjusted models (that also mutually adjusted for indoor and outdoor LTPA), the association was only statistically significant among cancer survivors who engaged in outdoor LTPA (5.83 nmol/L, 95% CI: 1.64 to 10.01). The interaction between indoor and outdoor activities was not significant (P-value=0.29). Analyses using logistic regression models were supportive. Our findings were similar when we classified activities that could be either indoor or outdoor (e.g., bicycling, swimming) as outdoor activities (6.39 nmol/L, 95% CI: 2.85-9.94), and classifying these activities as half-half (MET-in/week) to indoor and outdoor activities (7.26 nmol/L, 95% CI: 2.88-11.64) (Data not shown).

Likewise, we observed similar results in sensitivity analyses using BMI as a continuous variable; higher 25-OHD levels were associated with LTPA in the overall analyses (7.74 nmol/L, 95% CI: 4.53-10.95), and among those who engaged in outdoor LTPA (5.82 nmol/L, 95% CI: 1.69-9.95) (Data not shown). In stratified analyses, associations of LTPA with higher circulating 25-OHD levels was retained in the obese group in the 2001-2006 data (7.10 nmol/L, 95% CI: 2.51 to 11.70, outdoor LTPA) as well as 2007-2010 data (13.91 nmol/L, 95% CI: 3.86-23.96, overall LTPA) (Data not shown). Outdoor LTPA was lower in Non-Hispanic Black (69.2% inactive vs. 51.5% inactive among Non-Hispanic Whites, and 43.2% inactive among Hispanics) (Data not shown).

[Insert Table 3 and Table 4]

Discussion

We observed that being physically active was associated with higher circulating 25-OHD levels in a nationally representative sample of cancer survivors. Further analyses showed that the elevated 25-OHD levels were only statistically significant among cancer survivors who engaged in outdoor physical activity.

To the best of our knowledge, this is the first study to evaluate the associations of physical activity with circulating 25-OHD levels in cancer survivors. Our findings are, however, similar to what has been reported among non-cancer participants enrolled in NHANES (1988-1994).[16] Scragg and Camargo reported a 9.6 nmol/L increase in 25-OHD levels among participants who engaged in outdoor LTPA compared to those who did not engage in outdoor LTPA. The increase in 25-OHD levels associated with outdoor LTPA is higher than what we observed in our study population (5.83 nmol/L higher 25-OHD). This could be due to the different ways LTPA was categorized. The most active group in their study translates to participating daily in outdoor activity, whilst only 5.6% (weighted proportion) of cancer survivors in our sample achieved this physical activity level. To compare at an equivalently active level, our findings of a 5.83 nmol/L increase in cancer survivors is similar to 6.1 nmol/L higher 25-OHD level in individuals who were at a similar activity level (engaged in 13-30 times outdoor LTPA per month) reported by Scragg and Camargo.[16] Data from trials have shown that each 40 IU of vitamin D consumed increases serum 25-OHD concentrations by 0.53 nmol/L in adults.[34] The recommended dietary vitamin D allowance for adults in the US is 600 IU, which is expected to increase circulating 25-OHD levels by 15 nmol/L. Thus, our findings (a 5.83 nmol/L increase) suggests that engaging in outdoor LTPA could provide

clinically meaningful increases in 25-OHD levels among cancer survivors. A more recent analysis using NHANES 2003-2006 data reported increasing level of 25-OHD associated with higher level of objectively measured moderate-to-vigorous physical activity, but the association was not stronger for outdoor LTPA compared to indoor when using self-reported data.[17]

It is unclear whether physical activity has direct or indirect effects on 25-OHD levels. Sun exposure is the major determinant of circulating 25-OHD levels, hence, it is possible that physical activity may indirectly impact 25-OHD levels through increased sun exposure associated with outdoor activity[7] among active individuals; yet few studies have measured activities specifically to outdoor, or able to adjusted for sun exposure.[16 17 35 36] On the other hand, physical activity may directly impact 25-OHD metabolism. Zittermann and colleagues[18] reported higher calcium absorption rates and plasma calcritrol levels in exercise-trained young men compared to age-matched sedentary controls. Similarly, in a small study, young males who underwent muscle-building exercise (indoor) for at least 1 year had higher circulating 25-OHD, Gla-protein, and 1,25-dihydroxyvitamin levels compared to age-matched controls who received constant daily diet same as the exercise group.[20] However, whether this mechanism operates in cancer survivors is unclear, because of the physiological, biological and behavioral alterations associated with cancer, and cancer treatment.[32]

We observed statistically significant higher circulating 25-OHD levels associated with outdoor, but not with indoor, LTPA in the mutually adjusted model. Nevertheless, no

statistically significant interaction between indoor and outdoor LTPA was observed. It is likely that LTPA influence 25-OHD via multiple pathways, possibly both an indirect effect due to sun exposure and a direct impact on 25-OHD metabolism. However this warrants further investigation using precise measures of physical activity[37] and taking into consideration sun exposure, and other vitamin D metabolites.

We observed that obese cancer survivors who were active had higher circulating 25-OHD levels. Obesity is believed to induce low circulating 25-OHD levels through volumetric dilution of vitamin D in the excessive adipose tissue.[38] Given that obese cancer survivors are at higher risk of vitamin D deficiency compared to the non-obese,[39] [40] present findings suggested engaging in physical activity might be particularly important to maintain or increase circulating 25-OHD levels among obese cancer survivors. Future studies are needed to confirm these findings using more precise measures of adiposity (e.g., body fat percentage) in a larger study population.

The association between LTPA and dietary vitamin D supplement use appeared to differ between 2001-2006 data (p=0.19) and 2007-2010 (p=0.03) data, although the prevalence of dietary vitamin D supplement use were similar in two study phases (51.4% vs. 51.5%). In the 2007-2010 data, active cancer survivors are more likely to report dietary vitamin D supplement use compared to inactive ones. Thus, the non-significant findings of LTPA and circulating 25-OHD levels could arise from the change in self-reported LTPA measures from 2001-2006 to 2007-2010 data.

The main strength of this analysis is pooling cancer survivors from a nationally representative adult sample in the US. We aggregated five waves' data and achieved a fairly sizeable sample. In addition, we controlled for a range of factors that are known to affect the circulating 25-OHD levels. Further, we were able to compare associations of LTPA with 25-OHD by outdoor and indoor LTPA, thereby providing further insights on the associations of LTPA with 25-OHD levels.

There are a number of limitations to this study. First, the cross-sectional nature of this study makes it impossible to determine a causal effect. The debate on whether vitamin D deficiency is a risk factor for mortality or an indicator of good health is ongoing.[41 42] It is possible that active cancer survivors were more active because of better health status, than those who were inactive. Thus, the higher 25-OHD levels in active cancer survivors might be an indicator of better overall health. Second, season, an important determinant of 25-OHD levels, was only available in two categories. Solar radiation, required for skin to synthesize vitamin D, is weaker in winter compared to summer. However, there were no statistically significantly differences between winter (Southern states) and summer (Northern states) 25-OHD levels in our study population, probably owing to the timing of blood collection in each region. The NHANES study collected blood samples in the Southern states during winter, and in the Northern states during summer. Third, we were not able to conduct analyses stratified by cancer type or time since diagnosis because of the limited number of individual cancers. Finally, physical activity was self-reported. Participants who received chemotherapy within last 4 weeks were excluded from blood collection within the NHANES study. Chemotherapy associated reduction of

circulating 25-OHD level has been documented previously.[43-45] Therefore our findings might not be generalizable to patients receiving chemotherapy.

Our findings of an association between LTPA and 25-OHD, that was stronger for outdoor LTPA compared to indoor LTPA has implications for public health recommendations in cancer survivors. Although the casual relationship of 25-OHD with cancer survival is yet unclear, strong evidence supports the benefits of physical activity in improved cancer survival and the quality of life during survival.[37 46] Our findings suggest that 25-OHD might be a surrogate marker of physical activity that accounts for the direct and indirect effects of LTPA, particularly outdoor.[7 16] The proportion of cancer survivors in NHANES who did not engage in any LTPA was high, especially in the 2007-2010 (53.3%) compared to the 2001-2006 wave (38.3%). This observed decline in LTPA might be attributed to the differences in measures and may not reflect an actual change in LTPA levels, i.e. the 2001-2006 measure is comprised of 48 activity items whilst the 2007-2010 measure queries general physical activity participation. This differences in measures may also contribute to the non-significant findings observed in the 2007-2010 data. In fact, an increase in the physical activity level in the US population from 2001 to 2011 has been reported from the BRFSS data,[47] though this trend may not hold true in cancer survivors. Guidelines from the American Cancer Society[32] and American College of Sports Medicine[48] suggest that cancer survivors should follow the physical activity guidelines for Americans with specific exercise programming adaptations based on disease- and treatment-related adverse effects. However, physical activity levels in these populations are critically low during and after

treatment.[49] Physical activity interventions in cancer survivors may consider including early morning (before 11 am) outdoor activities for about 15 minutes. Notably, given the well-documented differences in cancer prognosis between non-Hispanic Blacks and other racial groups, and the emerging associations of vitamin D with cancer prognosis, physical activity interventions incorporating outdoor activities might be particularly important for cancer survival among non-Hispanic Blacks.

In conclusion, physical activity, particularly outdoor physical activity is associated with higher 25-OHD levels in cancer survivors. This adds to the potential health benefits of being physically active. Non-Hispanic Black cancer survivors, who are more likely to have vitamin D deficiency, were less likely to engage in outdoor LTPA. In view of the possible beneficial effects of vitamin D on cancer prognosis, engaging in outdoor physical activity could provide clinically meaningful increases in 25-OHD levels among cancer survivors.

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Table 1. Socio-demographic Characteristics and Leisure Time Physical Activity of Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2006), by Circulating 25-OHD levels (n=793)

			Circulating 25-OHD (nmol/L)					
		Overall	Quintile 1 (9.1-44.7)	Quintile 2 (45.9-56.8)	Quintile 3 (58.1-66.8)	Quintile 4 (68-80.3)	Quintile 5 (81-156)	P-value
2001-2006	N	793	208	160	143	153	129	
Age (year)	Mean (s.e.)	60.3 (0.6)	60.1 (1.5)	59.4 (1.8)	61.0 (1.6)	61.9 (1.4)	57.6 (1.6)	0.36
BMI								<.001
<18.5	%	1.9	1.9	0.2	1.8	2.6	3.4	
18.5 – 24.9	%	32.7	29.3	19.4	33.8	35.5	47.1	
25.0 – 29.9	%	32.1	23.2	36.1	38.4	31.2	32.1	
≥ 30	%	33.3	45.6	44.3	26.0	30.7	17.4	
Season								0.12
Winter (November to April)	%	34.3	43.2	38.8	31.0	26.1	31.5	
Summer (May to October)	%	65.7	56.8	61.2	69.0	73.9	68.5	
Sex								0.52
Male	%	32.7	29.2	32.5	33.3	39.4	38.6	
Female	%	67.3	70.8	67.5	66.7	60.6	70.4	
Race								<.001
Non-Hispanic white	%	86.1	72.1	81.9	90.9	93.8	93.6	
Non-Hispanic black	%	6.6	18.7	6.3	2.3	1.7	2.8	
Hispanic and other	%	7.3	9.2	11.8	6.8	4.5	3.6	
Smoking								0.06
Never smoked	%	39.1	32.5	42.7	48.7	36.1	36.3	
Former smoker	%	39.8	37.5	34.5	40.5	46.4	40.4	
Current smoker	%	21.1	30.0	22.8	10.8	17.5	23.3	
Vitamin D supplement use								<.001
No	%	48.6	75.8	52.7	34.8	42.5	34.3	
Yes	%	51.4	24.2	47.3	65.3	57.5	65.7	
Leisure time physical activity (LTPA)								0.001

Inactive	%	38.2	55.5	40.7	36.6	30.8	26.1	
Insufficiently Active	%	33.0	27.4	35.4	29.1	39.8	33.0	
Sufficiently Active	%	28.8	17.1	23.9	34.3	29.4	40.9	
Indoor LTPA								0.08
Inactive	%	61.7	70.3	67.4	53.8	61.2	54.2	
Insufficiently Active	%	18.2	15.3	20.1	21.5	17.4	16.7	
Sufficiently Active	%	20.1	14.4	12.5	24.7	21.4	29.1	
Outdoor LTPA								<.001
Inactive	%	52.0	72.3	51.2	54.7	39.4	41.5	
Insufficiently Active	%	22.0	12.9	24.1	15.8	29.9	27.5	
Sufficiently Active	%	26.0	14.8	24.7	29.5	30.7	31.0	

Table 2. Socio-demographic Characteristics and Leisure Time Physical Activity of Cancer Survivors Aged 20 years or Older from the NHANES (2007- 2010), by Circulating 25-OHD level (n=737)

			Circulating 25-OHD (nmol/L)					
		Overall	Quintile 1 (13.2-49.2)	Quintile 2 (49.3-63.9)	Quintile 3 (64.3-76.5)	Quintile 4 (76.6-93.4)	Quintile 5 (93.9-206)	P-value
2007-2010	N	737	194	153	139	143	108	
Age (year)	Mean (s.e.)	60.8 (0.7)	58.9 (1.3)	59.8 (1.1)	61.7 (1.4)	64.3 (1.5)	59.3 (2.0)	0.35
BMI								0.008
<18.5	%	2.0	2.2	0.6	1.5	1.8	3.9	
18.5 – 24.9	%	27.2	23.1	20.3	21.2	36.7	34.6	
25.0 – 29.9	%	34.0	24.7	45.5	34.1	30.4	35.6	
≥ 30	%	36.8	50.0	33.6	43.2	31.1	25.9	
Season								0.1
Winter (November to April)	%	32.6	39.7	32.7	34.2	22.4	33.9	
Summer (May to October)	%	67.4	60.3	67.3	65.8	77.6	66.1	
Sex								0.40
Male	%	37.8	29.3	42.8	41.2	39.9	36.2	
Female	%	62.2	70.7	57.2	58.8	60.1	63.8	
Race								<.001
Non-Hispanic white	%	82.6	57.3	81.9	88.8	91.5	94.1	
Non-Hispanic black	%	8.2	20.9	7.5	5.3	4.9	2.2	
Hispanic and other	%	9.2	21.8	10.6	5.9	3.6	3.7	
Smoking								0.03
Never smoked	%	47.5	48.5	55.1	48.9	43.1	43.8	
Former smoker	%	35.1	26.2	25.8	43.3	43.0	37.2	
Current smoker	%	17.4	25.3	19.1	9.8	13.9	19.0	
Vitamin D supplement use								<.001
No	%	48.5	81.8	61.1	46.1	32.8	20.0	
Yes	%	51.5	18.2	38.9	53.9	67.2	80.0	
Leisure time physical activity (LTPA)								0.04

Inactive	%	53.3	70.8	51.7	51.3	50.9	41.6
Insufficiently Active	%	16.6	12.6	20.8	15.7	14.3	19.8
Sufficiently Active	%	30.1	16.6	27.5	33.0	34.8	38.6

Table 3. Associations between Leisure-Time Physical Activity and Circulating 25-OHD level from Unadjusted and Multivariable Linear Regression models among Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2010).

2001-2006* (n=793)		Circulating 25-OHD (nmol/L)	
		Unadjusted Beta-coefficient (95% CI)	Adjusted † Beta-coefficient (95% CI)
Model 1:	Leisure time physical activity (LTPA)		
	Inactive	reference	reference
	Insufficiently Active	7.36 (2.65 to 12.07)	3.63 (-0.69 to 7.95)
	Sufficiently Active	12.16 (7.29 to 17.04)	8.07 (4.63 to 11.52)
	P for trend	<.001	<.001
Model 2:	Outdoor physical activity		
	Inactive	reference	reference
	Insufficiently Active	9.10 (5.15 to 13.04)	6.17 (1.74 to 10.59)
	Sufficiently Active	8.84 (4.16 to 13.52)	5.83 (1.64 to 10.01)
	P for trend	<.001	0.005
	Indoor physical activity		
	Inactive	reference	reference
	Insufficiently Active	3.15 (-1.63 to 7.94)	-1.22 (-4.97 to 2.52)
	Sufficiently Active	8.22 (2.50 to 13.93)	2.93 (-1.80 to 7.66)
	P for trend	0.004	0.23
2007-2010* (n=737)		Circulating 25-OHD (nmol/L)	
		Unadjusted Beta-coefficient (95% CI)	Adjusted † Beta-coefficient (95% CI)
Model 3:	Leisure time physical activity (LTPA)		
	Inactive	reference	reference
	Insufficiently Active	8.80 (-2.67 to 20.26)	5.70 (-4.19to 15.6)
	Sufficiently Active	12.04 (5.24 to 18.84)	5.73 (-1.68 to 13.15)
	P for trend	0.001	0.11

*Leisure-time physical activity (LTPA) data analyzed separately due to the changes in self-reported LTPA measures from wave 2005 - 2006 to 2007-2008.

†Adjusted for age, sex, race, body mass index, smoking status and dietary vitamin D supplement use.

Table 4. Associations between Leisure-Time Physical Activity and Circulating 25-OHD level from Unadjusted and Adjusted Logistic Regression models among Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2010).

2001-2006*	Circulating 25-OHD >=50 nmol/L (n=534)	
Reference:	Unadjusted	Adjusted‡
Circulating 25-OHD <50 nmol/L (n=259)	Prevalence ratio (95% CI) †	Prevalence ratio (95% CI) †
Model 1: Leisure time physical activity (LTPA)		
Inactive	reference	reference
Insufficiently Active	1.19 (1.02 to 1.33)	1.10 (0.88 to 1.27)
Sufficiently Active	1.36 (1.30 to 1.45)	1.32 (1.19 to 1.41)
P for trend	<.001	<.001
Model 2: Outdoor physical activity		
Inactive	reference	reference
Insufficiently Active	1.21 (1.10 to 1.30)	1.16 (1.01 to 1.27)
Sufficiently Active	1.24 (1.11 to 1.33)	1.22 (1.06 to 1.32)
P for trend	0.001	0.009
Indoor physical activity		
Inactive	reference	reference
Insufficiently Active	1.19 (0.99 to 1.33)	1.10 (0.87 to 1.27)
Sufficiently Active	1.21 (1.05 to 1.33)	1.07 (0.88 to 1.23)
P for trend	0.006	0.32
2007-2010*	Circulating 25-OHD >=50 nmol/L (n=531)	
Reference	Unadjusted	Adjusted †
Circulating 25-OHD <50 nmol/L (n=206)	Prevalence ratio (95% CI)	Prevalence ratio (95% CI)
Model 3: Leisure time physical activity (LTPA)		
Inactive	reference	reference
Insufficiently Active	1.15 (0.97 to 1.26)	1.14 (0.92 to 1.27)
Sufficiently Active	1.22 (1.07 to 1.30)	1.13 (0.90 to 1.27)
P for trend	0.008	0.18

*Leisure-time physical activity (LTPA) data analyzed separately due to the changes in self-reported LTPA measures from wave 2005 - 2006 to 2007-2008.

† Prevalence ratio and 95% confidence intervals were corrected using prevalence odds ratio and prevalence of high 25-OHD level

(>=50 ol/L) in reference groups.
‡Adjusted for age, sex, race, body mass index, smoking status and dietary vitamin D supplement use.

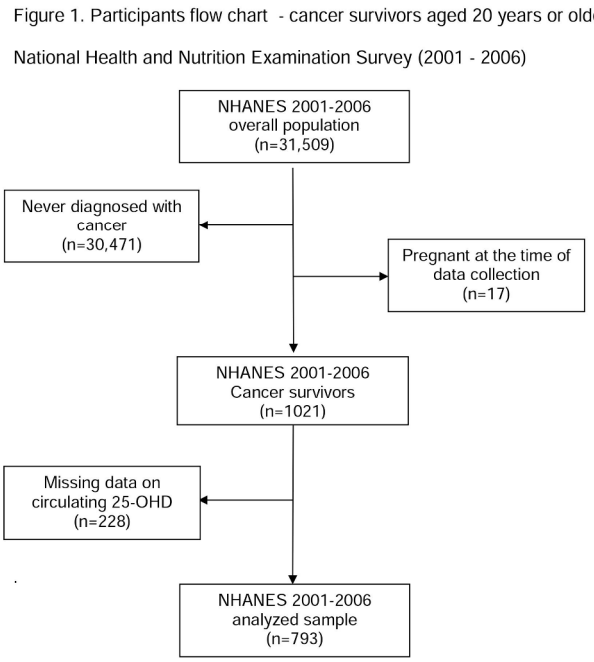

Figure 1. Participants flow chart - cancer survivors aged 20 years or older from the National Health and Nutrition Examination Survey (2001 - 2006)

215x279mm (300 x 300 DPI)

Figure 2. Participants flow chart - cancer survivors aged 20 years or older from the National Health and Nutrition Examination Survey (2007-2010).

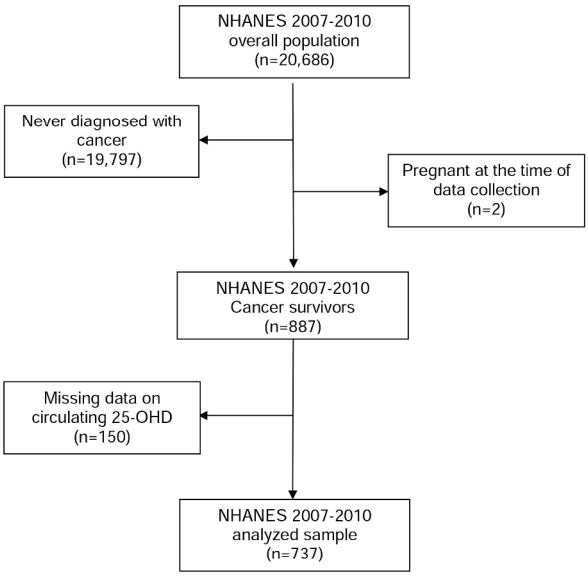

Figure 2. Participants flow chart - cancer survivors aged 20 years or older from the National Health and Nutrition Examination Survey (2007-2010)

215x279mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (page 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 5)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 6)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 6)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (Page 6)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 7-10)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 7-10)
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at (Page 6, Figures 1 and 2)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page 7-11)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 11-12)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 11-12)
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (Page 11)
		(*e*) Describe any sensitivity analyses (Page 12)

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Page 13)
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram (Figures 1 and 2)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 13)
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures (Page 13)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Page 13-14)
		(*b*) Report category boundaries when continuous variables were categorized (Page 7-10)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Page 14)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 16)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 19)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 16-18)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 19-20)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 22)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Leisure-time physical activity and circulating 25-hydroxyvitamin D levels in cancer survivors, a cross-sectional analysis using data from the US National Health and Nutrition Examination Survey

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Title: Leisure-time physical activity and circulating 25-hydroxyvitamin D levels in cancer survivors, a cross-sectional analysis using data from the US National Health and Nutrition Examination Survey

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Abstract: 294 words

Text: 3,912 words

Abstract

Objectives: Circulating 25-hydroxyvitamin D (25-OHD) is associated with improved cancer prognosis in some studies, yet it may be a surrogate marker for physical activity. We investigated the associations of leisure-time physical activity (LTPA) with circulating 25-OHD levels in cancer survivors, and determined whether associations differ by indoor and outdoor activity.

Design: Cross-sectional study.

Setting: The US National Health and Nutrition Examination Survey (NHANES).

Participants: Cancer survivors with available data on demographic information, measures of adiposity, smoking history, self-reported LTPA, circulating 25-OHD levels in five waves of NHANES (2001-2010).

Main outcomes measures: Circulating 25-OHD levels.

Results: Multivariable linear regression and logistic regression models were used to evaluate the associations of self-reported LTPA with 25-OHD, adjusting for potential confounders. Due to the differences in LTPA measure, the analyses were conducted separately for 2001-2006, and 2007-2010 data. We further estimated associations by indoor and outdoor activity in the 2001-2006 data. There were 1,530 cancer survivors (mean age=60.5 years, mean BMI=28.6 kg/m^2). The prevalent cancer sites were breast (19.3%), prostate (18.8%), cervix (10.4%), and colon (8.6%). Compared to inactive cancer survivors, being physically active was associated with higher circulating 25-OHD levels (8.07 nmol/L, 95%CI: 4.63 to 11.52) for 2001-2006 data. In the mutually adjusted model, higher outdoor activity (5.83 nmol/L, 95%CI: 1.64 to 10.01), but not indoor activity (2.93 nmol/L, 95%CI: -1.80 to 7.66), was associated with statistically significant

higher 25-OHD levels. The interaction between indoor and outdoor activities was, however, not significant (P-value=0.29). The only statistically significant association seen in the 2007-2010 data was among obese cancer survivors.

Conclusion: Physical activity, particularly outdoor activity is associated with higher 25-OHD levels in cancer survivors. In view of the possible beneficial effects of vitamin D on cancer prognosis, engaging in outdoor physical activity could provide clinically meaningful increases in 25-OHD levels among cancer survivors.

Strengths and limitations of this study

- To the best of our knowledge, this is the first study to investigate the association of leisure-time physical activity (LTPA) with circulating 25-hydroxyvitamin D (25-OHD) levels in cancer survivors. We further compared associations by outdoor and indoor LTPA.
- The current study pooled data from cancer survivors in a nationally representative adult sample in the US.
- This study controlled for a range of factors that are known to affect circulating 25-OHD levels.
- Study limitations includes (1), the cross-sectional nature makes it impossible to determine a causal association; (2) season, an important determinant of 25-OHD levels, was categorized into 2 (winter and summer, rather than winter, summer, fall and spring); (3) physical activity was self-reported.

Background

There are >15.5 million cancer survivors in the US and the number is expected to rise to 20 million by 2026.[1] Identifying factors, particularly modifiable factors, that improve prognosis and survival in this rapidly expanding demographic group is, therefore, a high priority.

There is emerging evidence that vitamin D status is associated with improved cancer prognosis and survival, particularly colorectal and breast cancers.[2-5] Circulating 25-hydroxyvitamin D (25-OHD) is the best indicator of overall vitamin D status because it has a long half-life, is unregulated by homeostatic systems in the body, and reflects total vitamin D from multiple determinants.[6-9] However, it has been suggested that circulating 25-OHD level may be a surrogate or biological marker for lifestyle factors that impact cancer prognosis, notably physical activity.[2 10 11] Physical activity, before and after cancer diagnosis, is associated with reduced mortality in cancer survivors,[12-14] although the underlying mechanisms are still being elucidated. In cancer-free population, leisure-time physical activity is associated with an increase in circulating 25-OHD levels; which is thought to reflect exposure to sunlight, a major determinant of circulating 25-OHD levels.[15] In support, studies have reported higher 25-OHD levels for the same amount of outdoor, compared to indoor physical activity,[16] although others have not.[17] Nevertheless, it has also been shown that physical activity and sun exposure may have independent effects on circulating 25-OHD levels, suggesting that indoor physical activity might be sufficient to increase circulating 25-OHD levels through its effect on 25-OHD metabolism, such as1,25-dihydroxyvitamin.[18-21]

To the best of our knowledge, no study has investigated the associations of physical activity with circulating 25-OHD levels in cancer survivors. Because physical activity declines after cancer diagnosis, findings in cancer-free population may not apply to cancer survivors. Using data from the National Health and Nutrition Examination Survey (NHANES), our objectives are to (i) investigate the associations of leisure-time physical activity with circulating 25-OHD levels in cancer survivors, (ii) determine whether associations differ by indoor and outdoor physical activity. Study findings could have implications for public health recommendations in cancer survivors because physical inactivity and vitamin D insufficiency are prevalent among cancer survivors.[22 23]

Methods

Study Population

The National Health and Nutrition Examination Survey (NHANES) was designed to provide cross-sectional estimates on the prevalence of health, nutrition, and potential risk factors among the civilian non-institutionalized U.S. population up to 85 years of age.[24] In brief, NHANES surveys a nationally representative complex, stratified, multistage, probability clustered sample of about 5,000 participants each year in 15 counties across the country. The NHANES obtained approval from the National Center for Health Statistics Research Ethics Review Board and participants provided written consent.

We extracted demographic information, measures of adiposity, smoking history, self-reported leisure time physical activity, circulating 25-OHD levels, cancer diagnosis, and combined them into a single dataset for each data collection wave. Participants were considered as cancer survivors if they answered “yes” to the question “Have you ever been told by a doctor or other health professional that you had cancer or a malignancy of any kind?” We excluded participants who had non-melanoma skin cancer. This interview question was only given to males and females 20 years or older, subsequently restricted the analysed sample to adult cancer survivors. We created a single dataset for each wave of data from NHANES in 2001 to 2002, 2003 to 2004, 2005 to 2006, 2007 to 2008, and 2009 to 2010, and excluded those who were never diagnosed with cancer, or were pregnant. (Figures 1 and 2)

Circulating 25-OHD levels

The process of blood collection is detailed in the NHANES Laboratory/Medical Technologist Procedures Manual.[25] Participants who received chemotherapy within last 4 weeks were excluded from blood collection in the NHANES study. Blood samples were collected, processed, stored and shipped to University of Washington, Seattle for testing. The lab method measuring 25-OHD for 2007-2010 changed from 2005-2006 and earlier in NHANES, and has been described previously.[26] Briefly, circulating 25-OHD concentrations were measured at the National Center for Environmental health, CDC, Atlanta, GA using the DiaSorin RIA kit (Stillwater, MN) between 2001 and 2006. We converted the 25-OHD data in 2001-2006 using provided regression to equivalent 25-OHD measurement from a standardized liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, which was used in the analysis of 25-OHD in NHANES 2007-2010 data. This standardization procedure therefore ensures that 25-OHD data is comparable between 2001-2006 and 2007-2010.

Socio-demographic characteristics

Socio-demographic characteristics including age, sex, race and ethnicity, and smoking status were extracted. Based on self-reported race and ethnicity, participants were classified into one of the three racial/ethnic groups: Non-Hispanic White, Non-Hispanic Black, and Hispanic and others. We classified participants into three groups: never smokers (did not smoke 100 cigarettes and do not smoke now), former smokers (smoked 100 cigarettes in life and do not smoke now), and current smokers (smoked 100 cigarettes in life and smoke now).

Body mass index (BMI)

Weight and height were measured at the time of physical examination in a mobile examination centre or in the participant's home. The measurements followed standard procedures and were carried out by trained technicians using standardized equipment. BMI was calculated as weight in kg/(height in meters)^2. We categorized study participants into standard BMI categories: underweight (<18.5kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0 – 29.9 kg/m^2), and obese (≥30.0 kg/m^2). For analytic purposes, we combined those who were underweight and those who had normal weight into 1 category (≤25 kg/m^2).

Season of blood draw

Blood samples were collected at the time of physical examination in a mobile examination center (MEC) or in the participants' home. Season of blood draw was determined from the documented month of physical examination. Months were reported in two groups: November 1st through April 30th, or May 1st through October 31st, and classified into winter or summer, respectively.[16]

Dietary Vitamin D supplement use

Information on dietary vitamin D supplement was retrieved from the 30-day Dietary Supplement dataset in the 2001-2006 and 2007-2010 data. In the 2001-2006 dataset, we obtained data on individual product for participants who reported taking vitamin supplement, and linked to the Dietary Supplements Ingredient Database.[27] Products'

ingredient that contained Vitamin D were aggregated for each participant, and then categorized into a binary variable (yes/no) for dietary vitamin D supplement use assessment. In 2007-2010 data, aggregated information on dietary supplement use (including vitamin D supplement use) was available, thus, was used to determine participants' dietary vitamin D supplement use (yes/no).

Self-reported leisure-time physical activity (LTPA)

The assessment on self-reported physical activity for 2007-2010 changed from 2005-2006 and earlier. There is no conversion provided between two assessments, therefore analyses for LTPA were conducted separately for the 2001 – 2006, and 2007 – 2010 data.

In the 2001-2006 data, participants self-reported specific LTPA in the past 30 days from a list of 48 activities, that if they engaged in certain activities, and the frequencies and durations of these activities. Each activity was coded into a metabolic equivalent task (MET) score based on the 2011 Compendium of Physical Activities, a valid and globally used instrument to quantify the energy expenditure of physical activity in adults.[28] For each reported activity, MET-minutes per week (MET-min/week) were calculated by multiplying the MET value of each reported activity by the minutes spent in the activity per seven days. Overall LTPA was summarized as the total MET-minutes per week of all reported activities.[29] Cancer survivors were classified as inactive (zero MET-min/week), insufficiently active (<750 MET-min/week), and sufficiently active (≥750 MET-min/week) based on the standard definition.[29] In addition, we categorized each of

the 48 listed activities into outdoor (e.g., walking, jogging, fishing) or indoor (e.g., aerobics, bowling, weights) activity. Activities that could be either indoor or outdoor (e.g., bicycling, swimming) were classified as indoor to ensure a conservative estimation of the associations between outdoor LTPA and 25-OHD. Both indoor and outdoor LTPA were summarized in MET-min/week, then classified as inactive (zero MET-min/week), insufficiently active (<450 MET-min/week), and sufficiently active (≥450 MET-min/week). A cutoff lower than 750 MET-min/week was used for indoor and outdoor activity, given they are sub-sets of overall LTPA. We used 450 MET-min/week as the cut-off given is the minimal goal of weekly LTPA.[29]

In the 2007-2010 data, participants self-reported their daily activities, leisure time activities, and sedentary activities, using questions based on the Global Physical Activity Questionnaire (GPAQ).[30] Levels of LTPA were calculated as the minutes per week that participants reported participating in moderate-to-vigorous-intensity physical activity (MVPA). Participants reported the number of days and minutes spent in moderate recreational and vigorous recreational activities in a typical week, by answering questions “In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational activities?”, “Minutes vigorous recreational activities”, “In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational activities?”, and “Minutes moderate recreational activities”. We summarized the total number of minutes for both activities, where the number of minutes spent in vigorous-intensity physical activity were doubled and added to the number of minutes of moderate-intensity physical activity to approximately equivalent the MET value.[31]

Cancer survivors were classified as inactive (zero min/week MVPA), insufficiently active (<150 min/week MVPA), and sufficiently active (≥150 min/week MVPA) based on the physical activity guidelines for cancer survivors.[32]

Statistical Analysis

Survey analysis procedures were used to account for the sample weights (MEC exam weight), stratification, and clustering of the complex sampling design to ensure nationally representative estimates. Information on socio-demographic characteristics, weight, height, season of blood draw, and self-reported LTPA was complete among cancer survivors who had available data on circulating 25-OHD levels. We calculated the descriptive statistics for participants' characteristics and LTPA categories by 25-OHD levels in quintiles separately in 2001-2006 data, and 2007-2010 data. We summarized weighted means and standard errors for continuous variables, and weighted proportions for categorical variables.

We estimated linear associations between LTPA and 25-OHD levels in both 2001-2006 and 2007-2010 data. The multivariable linear regression models for LTPA were adjusted for age, sex, race, BMI, smoking status, and season of blood draw. In the 2001-2006 data, we further estimated the linear associations between LTPA and 25-OHD separately by indoor and outdoor activities. Chi-square test indicated significant difference (P-value<0.001) between indoor and outdoor activities. In the multivariable linear regression models, we simultaneously adjusted for both activities. We tested for differences between the indoor and outdoor effects by including both in the regression model and testing for interaction. We examined the normality of residuals by kernel density estimate and standardized normal probability plots for all the linear regression models. Continuous 25-OHD data was categorized as low (<50 nmol/L) and high (≥50 nmol/L) 25-OHD based on definitions of vitamin D insufficiency.[30]

To calculate the prevalence ratios (PRs) of high 25-OHD level (≥50 nmol/L) across LTPA categories, we first calculated prevalence odds ratios (PORs) for each category in multivariable logistic regression models. Since the PORs do not approximate the PRs for common outcome (25-OHD ≥50 nmol/L), we used the baseline prevalence to correct the PORs and 95% confidence intervals based on existing method to obtain reliable PRs estimates.[33] We further conducted following sensitivity analyses: 1) using BMI as a continuous variable in the regression models; 2) stratification by BMI category; 3) classifying activities that could be either indoor or outdoor (e.g., bicycling, swimming) as outdoor activities; 4) classifying activities that could be either indoor or outdoor (e.g., bicycling, swimming) as half-half (MET-min/week) to indoor and outdoor activities.

All statistical significance was set at $p<0.05$. All statistical analyses were performed using Stata version 14.0 (STATA Corp., College Station, Texas, USA).

Results

Our study population consisted of 1,530 cancer survivors who had data on circulating 25-OHD levels. The most prevalent cancer sites were breast cancer (19.3%), prostate (18.8%), cervix (10.4%), and colon (8.6%). Participants' mean age at the time of baseline examination was 60.5 years, and their mean BMI was 28.6 kg/m^2. Circulating 25-OHD levels were significantly higher among those who reported dietary vitamin D supplement use than those who did not in both 2001-2006 (68.82 vs 56.74 nmol/L, p<.001) and 2007-2010 data (83.73 vs 60.88 nmol/L, p<.001). We observed statistically significant differences in circulating 25-OHD levels for most characteristics, except for age, and sex (Tables 1 (2001-2006) and 2 (2007-2010)). Cancer survivors who were obese, Non-Hispanic Black, or smokers had lower 25-OHD levels than those who had normal weight, Non-Hispanic White/Hispanic and were non-smokers, respectively.

[Insert Table 1 and Table 2]

Associations between LTPA and Circulating 25-OHD levels

Tables 3 and 4 summarize both the non-adjusted and adjusted associations between LTPA and circulating 25-OHD in linear regression and logistic regression models, respectively. Because LTPA measure differed between 2001-2006 and 2007-2010 and there is no conversion between the two, it is not possible to compare the findings between two study phrases directly. Cancer survivors who were sufficiently active had higher circulating 25-OHD levels than those who were inactive in univariate analyses, and these findings were maintained in multivariable analyses in the 2001-2006, but not the 2007-2010 data. This translated to 8.07 nmol/L (95% CI: 4.63 to 11.52) higher 25-

OHD levels in 2001-2006 phase in the multivariable-adjusted models. Furthermore, the comprehensive data on a list of 48 activities collected in 2001-2006 allowed us to extend the analyses to compare between indoor and outdoor LTPA in relation to 25-OHD levels. In the non-adjusted models (Table 3), higher levels of indoor and outdoor LTPA both were associated with higher 25-OHD levels. However, in multivariable-adjusted models (that also mutually adjusted for indoor and outdoor LTPA), the association was only statistically significant among cancer survivors who engaged in outdoor LTPA (5.83 nmol/L, 95% CI: 1.64 to 10.01). The interaction between indoor and outdoor activities was not significant (P-value=0.29). Analyses using logistic regression models were supportive. Our findings were similar when we classified activities that could be either indoor or outdoor (e.g., bicycling, swimming) as outdoor activities (6.39 nmol/L, 95% CI: 2.85-9.94), and classifying these activities as half-half (MET-in/week) to indoor and outdoor activities (7.26 nmol/L, 95% CI: 2.88-11.64) (Data not shown).

Likewise, we observed similar results in sensitivity analyses using BMI as a continuous variable; higher 25-OHD levels were associated with LTPA in the overall analyses (7.74 nmol/L, 95% CI: 4.53-10.95), and among those who engaged in outdoor LTPA (5.82 nmol/L, 95% CI: 1.69-9.95) (Data not shown). In stratified analyses, associations of LTPA with higher circulating 25-OHD levels was retained in the obese group in the 2001-2006 data (7.10 nmol/L, 95% CI: 2.51 to 11.70, outdoor LTPA) as well as 2007-2010 data (13.91 nmol/L, 95% CI: 3.86-23.96, overall LTPA) (Supplementary tables). The stratified analyses should, however, be interpreted cautiously because the relatively small number of participants in the different strata may not allow for very robust effect

estimates. Outdoor LTPA was lower in Non-Hispanic Black (69.2% inactive vs. 51.5% inactive among Non-Hispanic Whites, and 43.2% inactive among Hispanics) (Data not shown).

[Insert Table 3 and Table 4]

Discussion

We observed that being physically active was associated with higher circulating 25-OHD levels in a nationally representative sample of cancer survivors. Further analyses showed that the elevated 25-OHD levels were only statistically significant among cancer survivors who engaged in outdoor physical activity.

To the best of our knowledge, this is the first study to evaluate the associations of physical activity with circulating 25-OHD levels in cancer survivors. Our findings are, however, similar to what has been reported among non-cancer participants enrolled in NHANES (1988-1994).[16] Scragg and Camargo reported a 9.6 nmol/L increase in 25-OHD levels among participants who engaged in outdoor LTPA compared to those who did not engage in outdoor LTPA. The increase in 25-OHD levels associated with outdoor LTPA is higher than what we observed in our study population (5.83 nmol/L higher 25-OHD). This could be due to the different ways LTPA was categorized. The most active group in their study translates to participating daily in outdoor activity, whilst only 5.6% (weighted proportion) of cancer survivors in our sample achieved this physical activity level. To compare at an equivalently active level, our findings of a 5.83 nmol/L increase in cancer survivors is similar to 6.1 nmol/L higher 25-OHD level in individuals who were at a similar activity level (engaged in 13-30 times outdoor LTPA per month) reported by Scragg and Camargo.[16] Data from trials have shown that each 40 IU of vitamin D consumed increases serum 25-OHD concentrations by 0.53 nmol/L in adults.[34] The recommended dietary vitamin D allowance for adults in the US is 600 IU, which is expected to increase circulating 25-OHD levels by 15 nmol/L. Thus, our findings (a 5.83 nmol/L increase) suggests that engaging in outdoor LTPA could provide

clinically meaningful increases in 25-OHD levels among cancer survivors. A more recent analysis using NHANES 2003-2006 data reported increasing level of 25-OHD associated with higher level of objectively measured moderate-to-vigorous physical activity, but the association was not stronger for outdoor LTPA compared to indoor when using self-reported data.[17]

It is unclear whether physical activity has direct or indirect effects on 25-OHD levels. Sun exposure is the major determinant of circulating 25-OHD levels, hence, it is possible that physical activity may indirectly impact 25-OHD levels through increased sun exposure associated with outdoor activity[7] among active individuals; yet few studies have measured activities specifically to outdoor, or able to adjusted for sun exposure.[16 17 35 36] On the other hand, physical activity may directly impact 25-OHD metabolism. Zittermann and colleagues[18] reported higher calcium absorption rates and plasma calcritrol levels in exercise-trained young men compared to age-matched sedentary controls. Similarly, in a small study, young males who underwent muscle-building exercise (indoor) for at least 1 year had higher circulating 25-OHD, Gla-protein, and 1,25-dihydroxyvitamin levels compared to age-matched controls who received constant daily diet same as the exercise group.[20] However, whether this mechanism operates in cancer survivors is unclear, because of the physiological, biological and behavioral alterations associated with cancer, and cancer treatment.[32]

We observed statistically significant higher circulating 25-OHD levels associated with outdoor, but not with indoor, LTPA in the mutually adjusted model. Nevertheless, no

statistically significant interaction between indoor and outdoor LTPA was observed. It is likely that LTPA influence 25-OHD via multiple pathways, possibly both an indirect effect due to sun exposure and a direct impact on 25-OHD metabolism. However this warrants further investigation using precise measures of physical activity[37] and taking into consideration sun exposure, and other vitamin D metabolites.

We observed that obese cancer survivors who were active had higher circulating 25-OHD levels. Obesity is believed to induce low circulating 25-OHD levels through volumetric dilution of vitamin D in the excessive adipose tissue.[38] Given that obese cancer survivors are at higher risk of vitamin D deficiency compared to the non-obese,[39] [40] present findings suggested engaging in physical activity might be particularly important to maintain or increase circulating 25-OHD levels among obese cancer survivors. Future studies are needed to confirm these findings using more precise measures of adiposity (e.g., body fat percentage) in a larger study population.

The association between LTPA and dietary vitamin D supplement use appeared to differ between 2001-2006 data (p=0.19) and 2007-2010 (p=0.03) data, although the prevalence of dietary vitamin D supplement use were similar in two study phases (51.4% vs. 51.5%). In the 2007-2010 data, active cancer survivors are more likely to report dietary vitamin D supplement use compared to inactive ones. Thus, the non-significant findings of LTPA and circulating 25-OHD levels could arise from the change in self-reported LTPA measures from 2001-2006 to 2007-2010 data.

The main strength of this analysis is pooling cancer survivors from a nationally representative adult sample in the US. We aggregated five waves' data and achieved a fairly sizeable sample. In addition, we controlled for a range of factors that are known to affect the circulating 25-OHD levels. Further, we were able to compare associations of LTPA with 25-OHD by outdoor and indoor LTPA, thereby providing further insights on the associations of LTPA with 25-OHD levels.

There are a number of limitations to this study. First, the cross-sectional nature of this study makes it impossible to determine a causal associaiton. The debate on whether vitamin D deficiency is a risk factor for mortality or an indicator of good health is ongoing.[41 42] It is possible that active cancer survivors were more active because of better health status, than those who were inactive. Thus, the higher 25-OHD levels in active cancer survivors might be an indicator of better overall health. Second, season, an important determinant of 25-OHD levels, was only available in two categories. Solar radiation, required for skin to synthesize vitamin D, is weaker in winter compared to summer. However, there were no statistically significantly differences between winter (Southern states) and summer (Northern states) 25-OHD levels in our study population, probably owing to the timing of blood collection in each region. The NHANES study collected blood samples in the Southern states during winter, and in the Northern states during summer. Third, we were not able to conduct analyses stratified by cancer type or time since diagnosis because of the limited number of individual cancers. Finally, physical activity was self-reported. Participants who received chemotherapy within last 4 weeks were excluded from blood collection within the NHANES study. Chemotherapy

associated reduction of circulating 25-OHD level has been documented previously.[43-45] Therefore our findings might not be generalizable to patients receiving chemotherapy.

Our findings of an association between LTPA and 25-OHD, that was stronger for outdoor LTPA compared to indoor LTPA has implications for public health recommendations in cancer survivors. Although the casual relationship of 25-OHD with cancer survival is yet unclear, strong evidence supports the benefits of physical activity in improved cancer survival and the quality of life during survival.[37 46] Our findings suggest that 25-OHD might be a surrogate marker of physical activity that accounts for the direct and indirect effects of LTPA, particularly outdoor.[7 16] The proportion of cancer survivors in NHANES who did not engage in any LTPA was high, especially in the 2007-2010 (53.3%) compared to the 2001-2006 wave (38.3%). This observed decline in LTPA might be attributed to the differences in measures and may not reflect an actual change in LTPA levels, i.e. the 2001-2006 measure is comprised of 48 activity items whilst the 2007-2010 measure queries general physical activity participation. This differences in measures may also contribute to the non-significant findings observed in the 2007-2010 data. In fact, an increase in the physical activity level in the US population from 2001 to 2011 has been reported from the BRFSS data,[47] though this trend may not hold true in cancer survivors. Guidelines from the American Cancer Society[32] and American College of Sports Medicine[48] suggest that cancer survivors should follow the physical activity guidelines for Americans with specific exercise programming adaptations based on disease- and treatment-related adverse effects. However, physical activity levels in these populations are critically low during and after

treatment.[49] Physical activity interventions in cancer survivors may consider including early morning (before 11 am) outdoor activities for about 15 minutes. Notably, given the well-documented differences in cancer prognosis between non-Hispanic Blacks and other racial/ethnic groups, and the emerging associations of vitamin D with cancer prognosis, physical activity interventions incorporating outdoor activities might be particularly important for cancer survival among non-Hispanic Blacks.

In conclusion, physical activity, particularly outdoor physical activity is associated with higher 25-OHD levels in cancer survivors. This adds to the potential health benefits of being physically active. Non-Hispanic Black cancer survivors, who are more likely to have vitamin D deficiency, were less likely to engage in outdoor LTPA. In view of the possible beneficial effects of vitamin D on cancer prognosis, engaging in outdoor physical activity could provide clinically meaningful increases in 25-OHD levels among cancer survivors.

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Contributors: LY and ATT conceived and designed study, analysed and interpreted data, drafted and reviewed manuscript.

Data sharing statement: The NHNAES data are publically available at https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

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Table 1. Socio-demographic Characteristics and Leisure Time Physical Activity of Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2006), by Circulating 25-OHD levels (n=793)

			Circulating 25-OHD (nmol/L)					
		Overall	Quintile 1 (9.1-44.7)	Quintile 2 (45.9-56.8)	Quintile 3 (58.1-66.8)	Quintile 4 (68-80.3)	Quintile 5 (81-156)	P-value
2001-2006	N	793	208	160	143	153	129	
Age (year)	Mean (s.e.)	60.3 (0.6)	60.1 (1.5)	59.4 (1.8)	61.0 (1.6)	61.9 (1.4)	57.6 (1.6)	0.36
BMI								<.001
<18.5	%	1.9	1.9	0.2	1.8	2.6	3.4	
18.5 – 24.9	%	32.7	29.3	19.4	33.8	35.5	47.1	
25.0 – 29.9	%	32.1	23.2	36.1	38.4	31.2	32.1	
≥ 30	%	33.3	45.6	44.3	26.0	30.7	17.4	
Season								0.12
Winter (November to April)	%	34.3	43.2	38.8	31.0	26.1	31.5	
Summer (May to October)	%	65.7	56.8	61.2	69.0	73.9	68.5	
Sex								0.52
Male	%	32.7	29.2	32.5	33.3	39.4	38.6	
Female	%	67.3	70.8	67.5	66.7	60.6	70.4	
Race								<.001
Non-Hispanic white	%	86.1	72.1	81.9	90.9	93.8	93.6	
Non-Hispanic black	%	6.6	18.7	6.3	2.3	1.7	2.8	
Hispanic and other	%	7.3	9.2	11.8	6.8	4.5	3.6	
Smoking								0.06
Never smoked	%	39.1	32.5	42.7	48.7	36.1	36.3	
Former smoker	%	39.8	37.5	34.5	40.5	46.4	40.4	
Current smoker	%	21.1	30.0	22.8	10.8	17.5	23.3	
Vitamin D supplement use								<.001
No	%	48.6	75.8	52.7	34.8	42.5	34.3	
Yes	%	51.4	24.2	47.3	65.3	57.5	65.7	
Leisure time physical activity (LTPA)								0.001

Inactive	%	38.2	55.5	40.7	36.6	30.8	26.1	
Insufficiently Active	%	33.0	27.4	35.4	29.1	39.8	33.0	
Sufficiently Active	%	28.8	17.1	23.9	34.3	29.4	40.9	
Indoor LTPA								0.08
Inactive	%	61.7	70.3	67.4	53.8	61.2	54.2	
Insufficiently Active	%	18.2	15.3	20.1	21.5	17.4	16.7	
Sufficiently Active	%	20.1	14.4	12.5	24.7	21.4	29.1	
Outdoor LTPA								<.001
Inactive	%	52.0	72.3	51.2	54.7	39.4	41.5	
Insufficiently Active	%	22.0	12.9	24.1	15.8	29.9	27.5	
Sufficiently Active	%	26.0	14.8	24.7	29.5	30.7	31.0	

Table 2. Socio-demographic Characteristics and Leisure Time Physical Activity of Cancer Survivors Aged 20 years or Older from the NHANES (2007- 2010), by Circulating 25-OHD level (n=737)

			Circulating 25-OHD (nmol/L)					
		Overall	Quintile 1 (13.2-49.2)	Quintile 2 (49.3-63.9)	Quintile 3 (64.3-76.5)	Quintile 4 (76.6-93.4)	Quintile 5 (93.9-206)	P-value
2007-2010	N	737	194	153	139	143	108	
Age (year)	Mean (s.e.)	60.8 (0.7)	58.9 (1.3)	59.8 (1.1)	61.7 (1.4)	64.3 (1.5)	59.3 (2.0)	0.35
BMI								0.008
<18.5	%	2.0	2.2	0.6	1.5	1.8	3.9	
18.5 – 24.9	%	27.2	23.1	20.3	21.2	36.7	34.6	
25.0 – 29.9	%	34.0	24.7	45.5	34.1	30.4	35.6	
≥ 30	%	36.8	50.0	33.6	43.2	31.1	25.9	
Season								0.1
Winter (November to April)	%	32.6	39.7	32.7	34.2	22.4	33.9	
Summer (May to October)	%	67.4	60.3	67.3	65.8	77.6	66.1	
Sex								0.40
Male	%	37.8	29.3	42.8	41.2	39.9	36.2	
Female	%	62.2	70.7	57.2	58.8	60.1	63.8	
Race								<.001
Non-Hispanic white	%	82.6	57.3	81.9	88.8	91.5	94.1	
Non-Hispanic black	%	8.2	20.9	7.5	5.3	4.9	2.2	
Hispanic and other	%	9.2	21.8	10.6	5.9	3.6	3.7	
Smoking								0.03
Never smoked	%	47.5	48.5	55.1	48.9	43.1	43.8	
Former smoker	%	35.1	26.2	25.8	43.3	43.0	37.2	
Current smoker	%	17.4	25.3	19.1	9.8	13.9	19.0	
Vitamin D supplement use								<.001
No	%	48.5	81.8	61.1	46.1	32.8	20.0	
Yes	%	51.5	18.2	38.9	53.9	67.2	80.0	
Leisure time physical activity (LTPA)								0.04

Inactive	%	53.3	70.8	51.7	51.3	50.9	41.6
Insufficiently Active	%	16.6	12.6	20.8	15.7	14.3	19.8
Sufficiently Active	%	30.1	16.6	27.5	33.0	34.8	38.6

Table 3. Associations between Leisure-Time Physical Activity and Circulating 25-OHD level from Unadjusted and Multivariable Linear Regression models among Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2010).

2001-2006* (n=793)	Circulating 25-OHD (nmol/L)	
	Unadjusted Beta-coefficient (95% CI)	Adjusted † Beta-coefficient (95% CI)
Leisure time physical activity (LTPA)		
Inactive	reference	reference
Insufficiently Active	7.36 (2.65 to 12.07)	3.63 (-0.69 to 7.95)
Sufficiently Active	12.16 (7.29 to 17.04)	8.07 (4.63 to 11.52)
P for trend	<.001	<.001
Outdoor physical activity		
Inactive	reference	reference
Insufficiently Active	9.10 (5.15 to 13.04)	6.17 (1.74 to 10.59)
Sufficiently Active	8.84 (4.16 to 13.52)	5.83 (1.64 to 10.01)
P for trend	<.001	0.005
Indoor physical activity		
Inactive	reference	reference
Insufficiently Active	3.15 (-1.63 to 7.94)	-1.22 (-4.97 to 2.52)
Sufficiently Active	8.22 (2.50 to 13.93)	2.93 (-1.80 to 7.66)
P for trend	0.004	0.23
2007-2010* (n=737)	Circulating 25-OHD (nmol/L)	
	Unadjusted Beta-coefficient (95% CI)	Adjusted † Beta-coefficient (95% CI)
Leisure time physical activity (LTPA)		
Inactive	reference	reference
Insufficiently Active	8.80 (-2.67 to 20.26)	5.70 (-4.19 to 15.6)
Sufficiently Active	12.04 (5.24 to 18.84)	5.73 (-1.68 to 13.15)
P for trend	0.001	0.11

*Leisure-time physical activity (LTPA) data analyzed separately due to the changes in self-reported LTPA measures from wave 2005 - 2006 to 2007-2008.

†Adjusted for age, sex, race, body mass index, smoking status and dietary vitamin D supplement use.

Table 4. Associations between Leisure-Time Physical Activity and Circulating 25-OHD level from Unadjusted and Adjusted Logistic Regression models among Cancer Survivors Aged 20 years or Older from the NHANES (2001 - 2010).

2001-2006*	Circulating 25-OHD >=50 nmol/L (n=534)	
Reference:	Unadjusted	Adjusted‡
Circulating 25-OHD <50 nmol/L (n=259)	Prevalence ratio (95% CI) †	Prevalence ratio (95% CI) †
Leisure time physical activity (LTPA)		
Inactive	reference	reference
Insufficiently Active	1.19 (1.02 to 1.33)	1.10 (0.88 to 1.27)
Sufficiently Active	1.36 (1.30 to 1.45)	1.32 (1.19 to 1.41)
P for trend	<.001	<.001
Outdoor physical activity		
Inactive	reference	reference
Insufficiently Active	1.21 (1.10 to 1.30)	1.16 (1.01 to 1.27)
Sufficiently Active	1.24 (1.11 to 1.33)	1.22 (1.06 to 1.32)
P for trend	0.001	0.009
Indoor physical activity		
Inactive	reference	reference
Insufficiently Active	1.19 (0.99 to 1.33)	1.10 (0.87 to 1.27)
Sufficiently Active	1.21 (1.05 to 1.33)	1.07 (0.88 to 1.23)
P for trend	0.006	0.32
2007-2010*	Circulating 25-OHD >=50 nmol/L (n=531)	
Reference	Unadjusted	Adjusted †
Circulating 25-OHD <50 nmol/L (n=206)	Prevalence ratio (95% CI)	Prevalence ratio (95% CI)
Leisure time physical activity (LTPA)		
Inactive	reference	reference
Insufficiently Active	1.15 (0.97 to 1.26)	1.14 (0.92 to 1.27)
Sufficiently Active	1.22 (1.07 to 1.30)	1.13 (0.90 to 1.27)
P for trend	0.008	0.18

*Leisure-time physical activity (LTPA) data analyzed separately due to the changes in self-reported LTPA measures from wave 2005 - 2006 to 2007-2008.

† Prevalence ratio and 95% confidence intervals were corrected using prevalence odds ratio and prevalence of high 25-OHD level

(>=50 ol/L) in reference groups.
‡Adjusted for age, sex, race, body mass index, smoking status and dietary vitamin D supplement use.

Figure 1. Participants flow chart – cancer survivors aged 20 years or older from the National Health and Nutrition Examinination Survey (2001 – 2006)

Figure 2. Participants flow chart – cancer survivors aged 20 years or older from the National Health and Nutrition Examinination Survey (2007 – 2010)

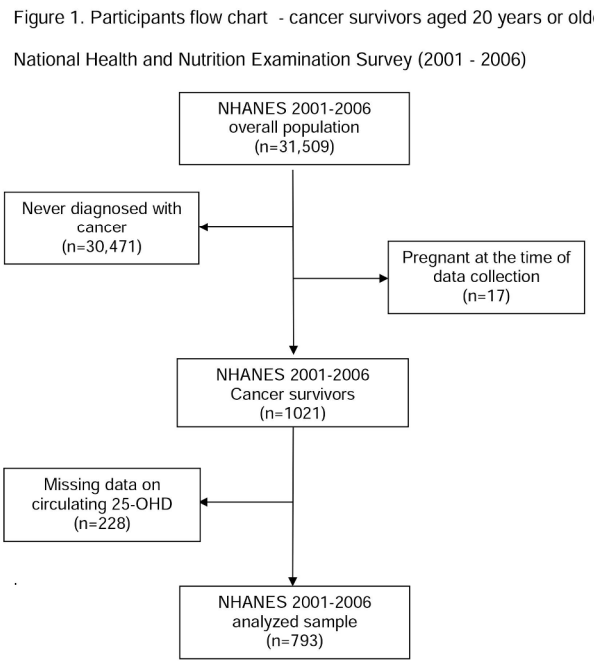

Figure 1. Participants flow chart - cancer survivors aged 20 years or older from the National Health and Nutrition Examination Survey (2001 - 2006)

215x279mm (300 x 300 DPI)

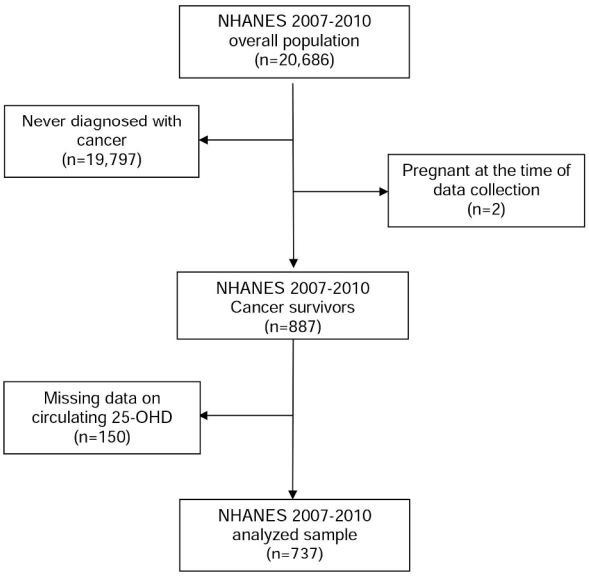

Figure 2. Participants flow chart - cancer survivors aged 20 years or older from the National Health and Nutrition Examination Survey (2007-2010)

215x279mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (page 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 5)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 6)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 6)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (Page 6)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 7-10)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 7-10)
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at (Page 6, Figures 1 and 2)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page 7-11)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 11-12)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 11-12)
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (Page 11)
		(*e*) Describe any sensitivity analyses (Page 12)

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Page 13)
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram (Figures 1 and 2)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 13)
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures (Page 13)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Page 13-14)
		(*b*) Report category boundaries when continuous variables were categorized (Page 7-10)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Page 14)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 16)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 19)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 16-18)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 19-20)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 22)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.